STUDIES ON SOME GASTRO-INTESTINAL PROTOZOAN PARASITES OF GOATS

By

DINESH JHA,

B. V. Sc. & A. H.,

Post Graduate College of Animal Sciences,

Indian Veterinary Research Institute,

IZATNAGAR (U. P.)

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G. Subramanian, M.Sc., Ph.D.
Assistant Professor
Lecturer in Parasitology

Post Graduate College of Animal Sciences
Indian Veterinary Research Institute
Izatnagar, U.P.

May 6 , 1964

This is to certify that the thesis entitled "Studies on some gastro-intestinal protozoan parasites of goats", embodies the work carried out by Shri Dinesh Jha under my supervision and guidance.

G. Subramanian

(G. Subramanian)

CONTENTS

INTRODUCTION

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The Goat (*Capra hircus* Linn.) has been called as the "poor man's cow" and truly so it forms an essential component in the economy of the Indian agriculturist. To an Indian farmer and villager, the goat is a constant companion and benefits him in many ways. Besides providing for the much needed source of animal proteins by way of milk and meat in the very low nutritional level of the diet in this country, the goat also provides a good supply of skin for varied purposes, mohair for its softness and warmth, and augments the organic manure supply for the much depleted soil.

The Indian Livestock Census of 1961 places the goat population in this country at 57 million, with Rajasthan having the largest population, followed by Maharashtra, Bihar and Uttar Pradesh. India stands fourth in the world's population of goats.

Goat meat comprises 36% of the total meat supply in this country. About 17 million of the goats are utilized annually for its meat, which is estimated to be 0.15 million tons (Nanporia, 1962-63).

There are six to eight breeds of the domestic goat in India. These are Beetal, Bikaneri, Konor, Bar-Bari, Berari, Marwari, Kathiawari and Jamnapari. The last breed is estimated to yield on an average nearly 272 kgs. of milk per lactation of about 250 days. Some selected goats in Punjab

and Uttar Pradesh are known to yield 2.7 - 3 kgs of milk per day.

About 21.3 million hides and skin from goats valued at Rupees 75 million are produced annually in this country and out of this Rupees 57.2 million is earned in foreign exchange by way of exports (Nanporia, 1962-63).

In India the maintenance and rearing of goats is very simple and does not entail heavy expenditure in as much as the goats are allowed to forage for themselves. Goats can maintain themselves on any rough herbage including plants which contain high percentage of tannic acids.

In spite of all the benefits accruing to the Indian farmer from the goats, very little is known or done for their better upkeep and protection from diseases. Nothing similar to the active and progressive British Goat Society exists in this country.

Very little is known about the occurrence and importance of protozoal infections amongst goats in India and this problem has not received any adequate attention.

Ray (1945, 1949) in recording the incidence of coccidial infections, enumerates and emphasizes on the importance of protozoal infections affecting sheep and goats in India. According to him, coccidiosis is a very important disease affecting lambs and kids and often causes death. E. arloingi (Marotel, 1905), Martin, 1909, causes 20-30% mortality in lambs and kids and this may go even upto 60-90% (Ray, 1949).

Ray (1949), however, has pointed out the utter lack of information with regard to protozoal infections in sheep and goats and suggested collection of data pertaining to incidence of coccidia and coccidiosis, determination of species involved, seasonal incidence and ecological factors controlling it and the breed susceptibility to coccidiosis.

Manjrekar (1954), Rao & Hiregaudar (1953-54), and Gill & Katiyar (1961) surveyed the incidence of coccidia in sheep and goats and their role in causing coccidiosis, "Coccidial dysentery" or "Red dysentery".

It is quite evident that coccidiosis could be a serious hazard to raising of lambs and kids. The disease could run a course of acute, subacute, chronic, or latent infection and various factors, such as physiological, climatological or biological, determine the degree of morbidity or mortality.

The species of Eimeria Schneider, 1875, are generally believed to be host specific though in the closely related sheep and goats hosts, this feature may not be evident. Species of Eimeria in sheep are usually considered to be infective to goats and vice versa. There are, however, no records of successful cross-transmission studies in goats. There is thus a necessity to determine the relationship of the ovine and caprine forms of coccidia.

Lambs and kids show retardation of growth, lose weight, the quality of wool goes down, there is decreased lactation, and there is a general loss of the economically useful

products and by-products. Foster (1949) estimates the annual loss due to coccidiosis in sheep of U.S.A. at 1.5 million dollars.

In the gastro-intestinal tract of sheep and goats, there is mention of another protozoan parasite, viz. Globidium Flesch, 1883 whose incidence has also been recorded in this country. Very little is known regarding this parasite and its taxonomic position has undergone frequent changes. Richardson & Kendall (1963) place it amongst the unclassified protozoan parasites.

The present work was undertaken to survey the incidence of some protozoan parasites in the gastro-intestinal tract of goats. This opportunity has been availed to do a detailed study and make a biometrical analysis of the different species of Eimeria in goats, to study the host specificity of one or more of the Eimeria species by cross-transmission work and study the incidence, morphology and histochemistry of Globidium species. Attempts have been made to study the host specificity of E. faurei (Moussu & Marotel, 1902) Martin 1909, and E. ninakohlyakimovae Yakimoff & Rastegaieff, 1930 in the caprine and ovine hosts. Cross-transmission experiments were set up with sporulated oocysts derived from goat and fed to young lambs.

REVIEW OF LITERATURE

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Eimeria Schneider, 1875

Morphology and taxonomy:

The parasites described under the genus *Eimeria* are coccidia characterised by ripe oocysts having four sporocysts with two sporozoites in each. Variations in the size, shape, colour and nature of the oocyst wall, the sporulation time, the presence or absence of oocyst residuum, sporocyst residuum, steida body and polar cap and the nature of the micropyle, in addition to host specificity and other biological features, are taken into consideration in differentiating the various species of *Eimeria*. Levine (1961) computes the number of morphological variations which are possible in a permutation and combination of the above characters at 2,654,208.

Credit for discovering coccidial parasites should probably go to Leeuwenhoek (1674) as is evident from his unpublished letters (Dobell, 1922). The first published and recognisable account of coccidial oocysts was by Carswell (1838) and Hake (1839). Both workers, however, gave wrong interpretation of what they saw. Later on, Kloss (1855), Eimer (1870), Schneider (1875), Schaudinn & Siedlecki (1897), Siedlecki (1898a, 1898b, 1898c, 1899) and Schaudinn (1900) gave descriptions of the various stages and the life cycle of *Eimeria* species and other related genera. The different

genera which were proposed and subsequently relegated as synonyms of Eimeria are:

Gregarina Eimer, 1870
Cytospermium Revolta, 1878
Psorospermium Rivolta, 1878
Coccidium Leuckart, 1879
Orthospora Schneider, 1881
Karyophagus Steinhaus, 1889
Cytophagus Steinhaus, 1891
Acystis Labbe, 1894
Pfeifferia Labbe, 1894
Bananella Labbe, 1895
Goussia Labbe, 1896
Crystallospora Labbe, 1896
Pfeifferella Labbe, 1899
Paracoccidium Laveran & Mesnil, 1902
Jarrina Leger & Hesse, 1922

A check list of the valid species in the genus Eimeria has been compiled by different workers from time to time (Levine & Becker, 1933; Becker, 1934; Hardcastle, 1943; Becker 1956; Orlov, 1956; Pellerdy, 1956). Eimeria species generally show a high degree of host specificity and this is reflected in the very large number of species which are on record. Pellerdy (1956) enumerates 393 valid species and a few more whose validity is doubtful.

In sheep and goats single or several mixed infections of Eimeria species have been described. Rivolta (1874) was the first person to record coccidial parasite in an ovine host. Subsequently, Leuckart (1879), Curtice (1892), Stiles

(1892), Maske (1893), Nocard (1893), Labbe (1899) and MacFadyean (1896) described the coccidial parasites in sheep under various names and most of these workers also drew attention to the pathology and pathogenesis of the infections.

Mazzanti (1900) was probably the first person, who gave the measurements of oocysts recovered from sheep in Italy. These oocysts have been subsequently shown to be those of E. faurei (Moussu & Marotel, 1902), Martin, 1909 (Yakimoff, Galouzo, Rastegaieff, Mizketwitsch & Tolstoff, 1926; Thomson & Hall, 1931). Thus, E. faurei was the first species to be described from an ovine host. Though it was first recorded in sheep, it has subsequently been described from the goats by various workers in different parts of the world. The detailed description of the oocysts of E. faurei (= Coccidium faurei) was given for the first time by Moussu & Marotel (1901, 1902) who also described its pathology and recorded observations similar to those of Curtice (1892), Stiles (1892), MacFadyean (1896) and Mazzanti (1900).

The first species to be recorded from goats was E. arloingi (Marotel, 1905), Martin, 1909. Subsequently this species has also been described from sheep and is now known to have world-wide distribution.

Many of the earlier workers had assumed that E. arloingi occurs in goats only while E. faurei is restricted to the sheep host. Baloset (1932a, 1932b, 1932c), from a detailed

review of these two species in sheep and goats, came to the conclusion that E. faurei and E. arloingi occur in both caprine and ovine hosts. Melikian (1955) and Orlov (1956) considered E. faurei to be a synonym of E. arloingi but this has not been accepted by the majority of workers (Becker, 1934; Christensen, 1938; Lotze, 1953; Rysavy, 1954; Pellerdy, 1956; Krilov, 1961; Levine, 1961; and Richardson & Kendall, 1963). There are reports of failure in cross-transmission of E. arloingi and E. faurei from goat and sheep to sheep and goat respectively (Balozet, 1932a; Krilov, 1961) and this gives sufficient justification for regarding E. arloingi and E. faurei as valid species.

Rudovsky (1922) described a new species of Eimeria in sheep and goats, viz., E. longispora and this has been included as a valid species in the check list of Hardcastle (1943) and Pellerdy (1956), though measurements of the oocysts and other details are not available. No subsequent worker has described E. longispora and Ershov (1956), Levine (1961) and Richardson & Kendall (1963) have not referred to this species in their books.

Spiegl (1925), in Germany, recorded a new species, E. intricata in sheep and gave the measurements of the oocysts as 42.0-50.4 x 30.6-36.0 µ. Sheather (1926), in England, described a new species in sheep, though he did not name it. From the descriptions available, this parasite is evidently E. intricata Spiegl, 1925. Subsequently, this

species has been recorded from America, Hungary, India and Russia (Kotlan, Mocsy & Vajda, 1929; Yakimoff, et al.,1926; Benbrook, 1933; Ray, 1949; Rao & Hiregaudar, 1953-54). The above mentioned records of E. intricata in America, England, Hungary, Germany and Russia are from ovine host only but the reports from India mention both ovine and caprine hosts.

E. parva Kotlan, Mocsy & Vajda, 1929, was the fourth new species to be described in sheep. Subsequently this has been recorded from sheep and goats in various parts of the world.

The fifth species of Eimeria to be discovered in goats was E. ninakohlyakimovae Yakimoff & Rastegaieff, 1930 (= E. ninae-kohl-yakimovae). This is now known to have cosmopolitan distribution.

Yakimoff & Rastegaieff (1930a) described E. galouzoi as a new species from goats in Russia. This has subsequently been relegated to the synonymy of E. parva and E. ninakohl-yakimovae in parts (Hardcastle, 1943; Pellerdy, 1956; Levine, 1961), though the Russian workers still regard E. galouzoi as a valid species.

Yakimoff (1931) described a new species, E. aemula from sheep in Russia, but subsequent workers have relegated it to the synonymy of E. arloingi (Becker, 1934; Hardcastle, 1943; Pellerdy, 1956; Richardson & Kendall, 1963). Levine (1961), however, synonymises E. aemula with E. faurei. Ershov (1956) considers E. aemula as valid.

Levine & Becker (1933) described 220 species under the genus Eimeria from 183 species of hosts and out of this 60 species were mammalian hosts. Becker (1934) enumerated five valid species in sheep and goats.

The sixth valid species to be recorded from the sheep Ovis arkhar and other related species was E. arkhari Yakimoff & Matschoulsky, 1937.

Christensen (1938) described two new species E. granulosa and E. pallida which he found in the faeces of sheep. E. pallida is described to differ from other species in the colour of the oocyst. E. granulosa n. s.p. is characterised by prominent shoulders at the micropyler end. Christensen (1938) follows Balozet (1932a) in accepting only five species of Eimeria in sheep and goats, in addition to the two new species described by him. E. pallida Christensen, 1938, has undergone frequent revision by other workers some of whom regard it as a valid species (Hardcastle, 1943; Levine, 1961; Richardson & Kendall, 1963) while others relegate it to the synonymy of E. parva (Pellerdy, 1956).

Honess (1942) described two new species in the Rocky Mountain bighorn sheep. These are E. ahsata (= E. ah-sa-ta) n. sp. and E. crandallis n. sp. Morgan & Hawkins (1952) and Lotze (1953) have questioned the validity of E. ahsata which is akin to the E. arloingi type of oocysts in sheep and goats. Smith, Davis & Bowman (1960), Levine (1961) and

Levine, Ivens, Smith & Davis (1962) have, however, indicated its distinctness from the other species. E. crandallis Honess, 1942, has been considered as a valid species (Hardcastle, 1943; Pellerdy, 1956; Levine, 1961 and Richardson & Kendall, 1963).

Hardcastle (1943) has provided a check list of the Eimeria species known upto his time and listed the following as valid species from sheep and goats. These are: E. ahsata, E. arkhari, E. arloingi, E. crandallis, E. faurei, E. granulosa, E. longispora, E. ninakohlyakimovae and E. pallida.

Landers (1952) described another new species in domestic sheep as E. honessi, which was, however, amended as E. punctata Lander 1955 to conform to the nomenclatorial rules. This species is distinguished by its size, presence of polar cap over a conspicuous micropyle and cone-shaped pits on the outer surface of the oocyst wall.

Ray (1952) recorded E. hawkensi n.sp. in sheep at Mukteswar-Kumaon, India. This new species was characterised by its size, the triangular polar cap, which appears to originate from the endocystic wall, and the very small micropyle opening. Strangely enough this species has not been reviewed by Pellerdy (1956) in his check list and Levine (1961) and Richardson & Kendall (1963) have not mentioned about this species. Reichenow (1953) created two subgenera in the genus Eimeria. These are Eimeria s.str.

and Eimeria (Globidium). Pellerdy (1956) recognised 10 species of Eimeria s. str. in sheep and goats and these are: E. ahsata, E. arkhari, E. arloingi, E. crandallis, E. faurei, E. granulosa, E. intricata, E. ninakohlyakimovae, E. parva, E. punctata, and E. pallida was synonymised with E. parva. Pellerdy (1956) followed Reichenow (1953) and relegated Globidium Flesch, 1883 as a subgenus of Eimeria.

Levine, Ivens & Fritz (1962) have recently described E. christenseni n.sp. from the faeces of a kid. The oocysts were ovoid and measured 34-41 x 23-28 μ with an average of 38 x 25 μ. The micropyle has a prominent mound shaped cap and the sporulated oocysts have sporocyst residuum but not oocyst residuum.

Levine (1961) recognised 11 valid species in sheep and goats. These are E. ahsata, E. arloingi, E. crandallis, E. faurei, E. gilruthi (= Globidium gilruthi), E. granulosa, E. intricata, E. ninakohlyakimovae, E. pallida, E. parva and E. punctata. Richardson & Kendall (1963) recognised more or less the same number of species as Levine (1961) except for E. gilruthi (= Globidium gilruthi) which has not been included under the genus Eimeria.

Prevalence and distribution:

The incidence of Eimeria species in goats has been recorded by many workers from different parts of the world but unfortunately it is not always clear from the literature

if the incidence is from both the caprine and ovine hosts or from one of them only. Many workers have generally assumed that the same species may affect both sheep and goats. This, however, remains to be proved for all species of Eimeria in sheep and goats.

E. arloingi was recorded in goats for the first time in France (Marotel, 1905). Balfour (1910) described two cases of coccidiosis in goats which accompanied him on the expedition to the White Nile but the species involved were not determined. Stevenson (1911) observed several cases of goat coccidiosis in Anglo-Egyptian Sudan and subsequently the reports of the Veterinary Services indicate high incidence and appreciable mortality in young kids. Velu (1919) in Morroco reported coccidiosis in goats caused by E.arloingi and the mortality rate was 25% in young suckling kids. Curasson (1921) reported coccidiosis in goats of Sudan and Senegal of the erstwhile French West Africa and the mortality rate was nearly 84%.

Schein (1921) described E. arloingi in goats of French Indo-China. Noller, Schurjohann & Vorbrodt (1922) while describing coccidiosis in sheep and goats, brought forth evidence to show that E. arloingi was the causative organism in both hosts. They compared the schizonts and oocysts in sheep with those from the goat and found the respective stages similar. Lotze (1953), however, questioned the observations of Noller et al. (1922) and showed that the schizonts described by them did not belong to E. arloingi.

Moller (1923) reported E. faurei from goats in the Berlin Zoological Garden. Reitsma (1923) encountered a mortality of 25% in the goats of Holland. Yakimoff (1930) described E. arloingi, E. ninakohlyakimovae and E. galouzoi from goats in the North Caucasia region of Russia. Balozet (1932a, 1932b) found 56% of goats infected with E. arloingi, 2% with E. faurei, 34% with E. ninakohlyakimovae and 22% with E. parva in Tunisia. Balozet (1932a, 1932b) considered all the five species, viz., E. arloingi, E. ninakohlyakimovae, E. faurei, E. parva and E. galouzoi to be common to sheep and goats.

Rogick (1938) found 100% of adult goats in Sao Paulo to be harbouring E. arloingi though the infection was mild and did not cause any morbidity or mortality. Van Volkenburg (1938) has indicated in his check list that only E. faurei, E. arloingi, and E. ninakohlyakimovae were found in the goats of Puerto Rico.

Jacobs (1943) in describing the incidence of caprine coccidiosis in Germany found 18% goats infected with E. arloingi and E. faurei and 9% with E. parva.

Ray (1945) recorded E. arloingi and E. faurei in the sheep and goats of India and subsequently (Ray, 1949) also added E. ninakohlyakimovae and E. intricata to the list. Ray (1949), however, did not indicate the incidence of these parasites in the caprine and ovine hosts separately.

Dikmans (1945) in the check list for North America enumerated E. arloingi, E. intricata, E. granulosa, E. pallida, E. parva, E. ninakohlyakimovae and E. ahsata to be present in sheep and goats. Morgan & Hawkins (1952) added E. faurei and E. grandallis to the check list of Dikmans (1945).

Sharma (1951-52) recorded E. arloingi, E. faurei, E. granulosa, E. ninakohlyakimovae, E. pallida and E. parva in goats of Punjab, while Rao & Hiregaudar (1953-54) found, in addition, E. intricata in the Bombay State. Rao & Hiregaudar (1953-54), however, have not indicated the incidence of these species in sheep and goats respectively.

Melikian (1955) found 34-100% of goats infected with Eimeria species in the Armenian Republic of USSR. Ershov (1956) recognised only E. faurei, E. arloingi, E. intricata, E. parva, E. ninakohlyakimovae, E. galouzoi and E. aemula as valid species in sheep and goats of Russia. Of these, E. arloingi was found to be the most pathogenic to goats. Krilov (1961) added E. ahsata to the list of Ershov (1956). Svanbaev (1957), while surveying goat coccidia in Western Kazakhstan, found E. arloingi in 52%, E. faurei in 40%, E. ninakohlyakimovae in 48% and E. faurei in 31% of the goats. In addition to these, E. galouzoi was also present.

In addition to the above mentioned records of the incidence and intensity of infection with Eimeria sp. in

goats, the following countries and regions have also recorded coccidiosis in goats. These are: Argentina (Ruppert, Rottgardt & Scasso, 1925), Austria (Rudovsky, 1922), Belgian Congo (Deom & Mortelmans, 1956), Brazil (Pacheo & Penha, 1929; Rogick, 1938; Torres, 1938; Torres & Ildefonso Ramos, 1938), Czechoslovakia (Rysavy, 1954), France (Martin, 1912), Germany (Martin, 1907; Karsten, 1913; Bausewein, 1921; Noller & Otten, 1921; Noller, 1923, 1924; Schurjohann, 1923; Schmidt, 1924), Holland (Nieschulz 1924a, 1924b), India (Baldrey, 1906; Manjrekar, 1954; Gill & Katiyar, 1961), Russia (Zion & Schtschenikoff, 1923; Yakimoff, 1927, 1930; Yakimoff & Rasteigaeiff, 1930a, 1930b; Shiyanov, 1954; Paichuk, Tsigankov & Zavadskaya, 1962), Sardinia (Deiana and Delitala, 1954), Switzerland (Vogt, 1930), South Africa (Fantham, 1919, 1923), Uraguay (Carballo Pou, Viera, Calzada & Rodriguez Garcia, 1938) and Venezuela (Mayadudon & Ayala Lopez, 1959-60).

Host specificity and cross transmission:

A review of the literature reveals the attempts which have been made to determine the degree and range of host specificity in Eimeria species. Bruce (1921) emphasized the strong host specificity of Eimeria species by his failure to infect sheep with oocysts obtained from cattle faeces. Dieben (1924) succeeded in infecting brown rats with E. miyairii Ohira, 1912 taken from black rat and

vice versa. Henry (1931) succeeded in establishing the transmission of Eimeria species [E. tenella (Railliet & Lucet, 1891) Fantham, 1909; E. acervulina Tyzzer, 1929 and E. mitis Tyzzer, 1929] from the domestic fowl to turkeys. These observations show that Eimeria species in closely related hosts does not exhibit a high degree of host specificity. In distantly related hosts or dissimilar hosts, the coccidial parasites show a high degree of host specificity (Galli-Valerio, 1918; Hall & Wigdor, 1918; Bruce, 1921; Andrews, 1927 and Becker, 1933). In analysing the causes, Andrews (1927) suggested that the digestive juices in unnatural host or hosts does not facilitate the excystation of sporulated oocysts.

Corcuff (1928) has cited the attempts of many workers who failed to establish E. faurei from sheep in goats and E. arloingi from goats in sheep. Balozet (1932a) failed in transmitting E. ninakohlyakimovae from caprine to ovine hosts. Becker (1934) attributed these failures to unknown causes though Balozet (1932a) considered the failure in cross transmission to be due to the advanced age of the lambs used.

Krilov (1961) set up cross transmission experiments for E. arloingi from sheep to goat and vice versa. He did not succeed in establishing clear cut infections in goats of 3 to 24 months age when even 100,000 sporulated oocysts of E. arloingi from sheep were used nor when

lambs, 1.5 months old, were infected with 210,000 oocysts of E. arloingi from goats. Krilov (1961) similarly failed to transmit E. ahsata from sheep to goat and from these experiments he concluded that the forms of Eimeria in sheep and goats are Xenodemes or biological races. Krilov (1961) named the ovine form as E. arloingi forma ovina. Krilov's (1961) work, however, is subject to some criticism in as much as it is not known whether the experimental animals used had picked up prior natural infection and hence developed immunity.

Sporulation:

One of the characteristics utilized in describing oocysts of Eimeria is the time required for sporulation under ordinary room temperature conditions. The sporulation time for E. arloingi has been variously given as 1-2 days (Christensen, 1938), 2-3 days (Morgan & Hawkins, 1952), 3-4 days (Balozet, 1932a), 3 days (Lerche, 1921; Becker, 1934) and 4 days (Ershov, 1956); for E. faurei, 1-2 days (Christensen, 1938) and 3-4 days (Balozet, 1932a; Becker, 1934; Ershov, 1956) for E. ninakohlyakimovae, 1-2 days (Christensen, 1938), 7 days (Becker, 1934; Ershov, 1956), 7-8 days (Balozet, 1932a); for E. parva 1-2 days (Christensen, 1938), 7 days (Becker, 1934; Ershov, 1956), 7-8 days (Balozet, 1932a); for E. intricata 3-5 days (Christensen, 1938; Levine, 1961), 9 days

(Sheather, 1926; Becker, 1934; Ershov, 1956); for E. aemula 3-4 days (Ershov, 1956); for E. pallida, 1 day (Christensen, 1938; Levine, 1961); for E. punctata, 1½-2 days (Landers,1952); for E. granulosa and E. galouzoi, 3-4 days (Ershov, 1956; Christensen, 1938), 4-8 days (Levine, 1961) and for E. hawkensi 10 hours at 37°C (Ray, 1952). The sporulation time for E. christenseni, E. ahsata, E. crandallis, E. longispora and E. arkhari are not available in the literature.

Globidium Flesch, 1883

This protozoan parasite was for the first time recorded from the small intestine of a horse (Equus caballus Linn.) in Germany and designated as Globidium leuckarti Flesch, 1883. Subsequently, this genus has been reported from several wild and domesticated animals in different parts of the world, as cutaneous, subcutaneous, abomasal or intestinal parasites. The taxonomic status of the cutaneous form of the parasite has undergone frequent changes and the general tendency now is to relegate it to another genus, viz., Besnoitia Franco & Borges, 1916 (Babudieri, 1932; Enigk, 1934; Reichnow, 1953; Pols, 1960). The validity of Besnoitia was established by Jellison (1956) and Frenkel (1953).

The intestinal form of Globidium parasite has been reported in equines (Flesch 1883, 1884; Henry & Masson, 1922; Hobmaier, 1922; Kupke, 1923; Navez, 1925; Reichnow, 1940; Hemmert-Halswick, 1943; Hiregaudar, 1956); in cattle (Balfour, 1910; Montgomery 1910; Muller, 1914; Van Nederveen, 1922; Pillers, 1928; Hasan, 1935); in camel (Henry & Masson, 1932; Enigk, 1934; Naville, 1936, Abdussalam & Rauf, 1958); in Kangaroo (Blanchard, 1885, Gilruth & Bull, 1912); in wombatt (Gilruth & Bull, 1912); in wallaby (Gilruth & Bull, 1912; Wenyon & Scott, 1925; Triffit, 1926a; Acton, cited by Knowles, 1928); in armadillo (da Cunha & Muniz, 1928;

da Cunha & Torres, 1924, 1926); in snakes (Guyenot, Naville & Ponse, 1922; Harrant & Cazal, 1934) and the doubtful records in Gobius minutus and Anoglossus grohmanni (Neumann, 1909). In sheep and goats, there are many records as indicated below. Globidium species has not been recorded from pigs, dogs or poultry.

Maske (1893) described forms similar to Globidium and considered them to be related to Gregarines. Moussu & Marotel (1901, 1902) considered them to be the developmental stages of Eimeria faurei (Moussu & Marotel, 1902) Martin, 1909 (= Coccidium faurei). Nocard (1893) described nodules in the stomach and intestine of sheep and considered them to be coccidia, but as pointed out by Yakimoff et al. (1926), he was dealing with a Globidium cyst.

Chatton (1910a, 1910b) created a new genus and species Gastrocystis gilruthi for the reception of Globidium parasite in sheep and goats but subsequently in the same year renamed it as Globidium gilruthi.

Noller (1920) reviewed the generic status of Globidium and the parasites under the related genera Gastrocystis Chatton, 1910; Ileocystis Gilruth & Bull, 1912; Lymphocystis, Gilruth & Bull, 1912; Sarcocystis Lankester, 1882; Balbiania Blanchard, 1885; Haplogastrocystis Chatton 1912; and Besnoitia Franco & Borges 1916, and relegated all these genera synonymy of Globidium Flesch, 1883.

Hobmaier (1922) and Ashworth (1923) regarded Globidium to be a fungi. Wenyon (1926) indicated its similarity to Rhinosporidium Minchin & Fantham, 1905, but classified the parasite under the order Sarcosporidia Butschli, 1882.

Triffit (1925, 1926b, 1928) did not find any evidence of cellulose in the cysts of Gastrocystis gilruthi (= G. gilruthi) and the contained spores could not be cultivated, though changes similar to that of the spores of Sarcocystis tenella Railliet 1886, were noticed when placed in 1% glucose solution. On the basis of these observations, she considered Gastrocystis gilruthi (= Globidium gilruthi) and Sarcosystis tenella to be relared and the latter being an aberrant form of the former. Pillers (1928), however, could not find any evidence to substantiate the close relationship of Globidium with coccidia or Sarcosporidia. Riechenow (1929) drew resemblance to the Gregarines rather than to Sarcosporodia.

Babudieri (1932) reviewed the morphology and taxonomic status of Globidium and the other related forms. He created a new order Globidia under the class Sporozoa Leuckart, 1879 and Subclass Sarcosporidia, Balbiani, 1885, and defined this new order as possessing cysts which are situated in the submucosa of the alimentary tract and where the parasite does not secrete a toxin. There are very few siderophilic granules in the spores, which are derived from blastophores, and the host cell is a hypertrophied somatic cell, the

nucleus of which has a marginal situation. In this order, the family Globididae Babudieri, 1932 was created to include Globidium and Ileocystis with the former genus to include all those species which are found as cysts either in the cutaneous tissue or in the mucosa of the gastro-intestinal tract.

Henry & Masson (1932) while describing a new species in camel, G. cameli, and a redescription of G. faurei (= G. gilruthi) in the intestine of sheep, defined these parasites as a large form of coccidia with large endogenous developmental stages and which result in the formation of large oocysts with thick walls formed by fusion of refractile corpuscles or globules.

Reichenow (1940) and Hiregaudar (1956) while describing the oocysts of G. leuckarti from the faeces of a donkey and a horse respectively found that, in the flotation technique, the oocysts of G. leuckarti did not rise to the surface due to its high specific gravity and hence escaped detection. Reichenow (1940) found ripe oocysts containing 4 sporoblasts with two sporozoites in each and unripe oocysts which sporulated in 2-3 weeks at 22-26°C. On the basis of these observations, Reichenow (1940, 1953) came to the conclusion that Globidium is similar to Eimeria and hence relegated the former as a subgenus of the latter. Reichenow (1953), however, pointed out the inadequacy of the diagnostic characters, lack of sharp demarcation

between the subgenera Eimeria (Globidium) and Eimeria s.str.

Neveu-Lemaire (1943) accepted Globidium as a valid genus and included both the intestinal and the cutaneous forms of the parasite in it but regarded G. faurei as the valid species in sheep and relegated G. gilruthi, E. intricata and E. arloingi to its synonymy.

Boughton (1942) observed white cyst-like bodies in the posterior half of the small intestine of bovines, resembling the schizonts of E. faurei as described by Moussu & Marotel (1902) and Chatton (1910) and the cysts of Globidium as described by Marsh & Tunnicliff (1941). He further found that these cyst-like bodies correspond to the schizont stages of E. bovis Zublin 1908, both in natural and in experimental infections. Hammond, Davis & Bowman (1944) and Hammond, Bowman, Davis & Simms (1946) brought forth some biological and histological evidence to show that the cyst-like bodies described as Globidium sp. are the sexual stages in the life cycle of Eimeria.

Richardson (1948) while describing coccidiosis in sheep and goats, considered it doubtful that G. gilruthi is a microgametocyte of E. intricata. The fact that oocysts are formed in G. cameli and the fact, that the trophozoite stages of E. faurei occur in the small intestine of sheep and is not associated with globidial type of cysts, has led Richardson (1948) to conclude that a cyst-like structure in the gastro-intestinal tract should

not be considered to belong to Eimeria unless controlled experimental work demonstrates a definite connection between the oocysts of the Eimerial type with the Globidial cysts.

Pellerdy (1956) in his catalogue of the genus Eimeria synonymised Globidium with the genus Eimeria in agreement with the views of Kotlan, Pellerdy & Versenyi (1951).

Pols (1960) has made a detailed review of the complicated nomenclatorial history of Globidium and the related genera. He reinstates Besnoitia as a valid genus following the opinion of Bennett (1933); Barrairon (1938); Cuille, Chelle & Berlureau (1936); and Frenkel (1956), but does not offer any suggestion regarding the validity of the intestinal form of Globidium species.

From the above review of literature pertaining to Globidial infections in the gastro-intestinal tract, it is evident that Globidium is a parasite with an undetermined life-cycle and hence imperfectly known in its taxonomic status. It would, however, appear to have a distinct affinity to Eimeria (Richardson & Kendall, 1963).

The incidence of Globidium infection in sheep and goats has been reported from Australia (Gilruth, 1910; Rae & Wilson, 1959), Egypt (Soliman, 1958), France (Nocard, 1893; Mousau & Marotel, 1902; Chatton, 1910); Germany (Maske, 1893); India (Tewari & Iyer, 1960); Japan (Isshiki, 1950); Pakistan (Sarwar, 1951); Turkey (Guralp & Urman,1957);

United States of America (Alicata, 1930; Marsh & Tunnicliff, 1941; Ferguson & Goldsby, 1961).

Maske (1893) described cysts, similar to Globidium, in the abomasum of sheep slaughtered at the Lubeck abattoir. Moussu & Marotel (1902) described these parasites from the abomasum of sheep and considered them to be the developmental stages of E. faurei.

Gilruth (1910) observed the parasite in the abomasal mucusm membrane of a sheep and described the condition as malignant transudation. He also gave a brief description of the parasite and found the sporozoites in the cyst to measure 4-6μ x 0.5μ and to possess flagella, but subsequent workers have not encountered such a structure. Chatton (1910) described the cysts of Globidium from the abomasum and the small intestine of sheep and goats and the contained spores, according to him, resembled those of Sarcosporidia Butschli, 1882.

Triffit (1925, 1928) examined sheep at random from the slaughter houses in London, and found 92% to be infected with G. gilruthi (= Gastrocystis gilruthi). The cysts were found only in the abomasum, with those in the mucosa being larger than those in the gastric glands. The cyst wall comprised of two concentric layers and was provided with a single large nucleus. Mature spores contained in the cysts measured 12μ x 2.5μ and were truncated at one end.

Alicata (1930) has recorded the incidence of this parasite in the abomasum of 8.9%, 10.8% and 8.3% of the sheep on different occasions. The cysts of this parasite were found to be white nodules located in the mucus lining of the abomasum.

Canham (1932) observed these parasites in the mucus membrane of the abomasum of sheep and the cysts of the size of a pinhead were filled with greenish or yellowish fluid. The contained spores, 10/u long were cigar shaped with both the ends tapering, and staining well with Giemsa stain.

Marsh & Tunnicliff (1941) found the principal lesions in the small intestine which was studded with white cysts. These cysts were rare in the abomasum, fewer in the duodenum but the number increased in the small intestine. The lanceolate spores, 10/u long showed no movement, when released from the cyst wall. Coccidial oocysts were also found as concurrent infection in the scrapings of the ileum and rectum.

Sarwar (1951) reported this parasite from the mucus membrane of the abomasum in 34% and 93.8% of the sheep and goats, respectively. Guralp & Urman (1957) found cysts of G. gilruthi in the cardiac and fundus region of the abomasum in 98.18% of the sheep. Soliman (1958) found the cysts in the fundus region of abomasum and also in the

jejunum and ileum of 18% of sheep and 28% of goats. Rao & Wilson (1959) found a greater percentage of the cysts in the abomasum and very few in the caecum of Merino sheep. Soliman (1960) observed the cysts in 32% of sheep and 40% goats. Tewari & Iyer (1960) recorded and described the presence of Globidium cysts in the small intestine of three goats. Ferguson & Goldsby (1961) found the cysts in the abomasum of 17.27% of the goats.

MATERIALS AND METHODS

MATERIAL AND METHODS

For this work the viscera of freshly slaughtered goats, in addition to faecal samples from the rectum of the experimental animals, were utilized. The goats were slaughtered for harvesting the spleen in connection with the mass production of freeze-dried rinderpest vaccine at the Indian Veterinary Research Institute, Izatnagar. These goats were purchased through a contractor and came from different places in Uttar Pradesh, Delhi, Rajasthan, Bihar, Madhya Pradesh and Himachal Pradesh. The goats had lived under different climatic conditions in the mountainous region, submountainous region and the Gangetic plains of this country. There was thus a good opportunity to have materials from a wide area. The goats, which are slaughtered, hardly spend 7-11 days on arrival and hence the protozoan fauna represents natural infection picked up from their source of origin or during transit. The goat population available for this work comprised of both sexes and the average age ranged from 1-3 years.

The viscera of the goats were collected at random and on an average 10-20 gastro-intestinal tracts from the abomasum to the rectum were examined daily for the presence of oocysts of Eimeria and Globidium and their tissue stages.

Both the ends of the gastro-intestinal tracts were tied before being brought to the laboratory for examination. Each sample of the alimentary canal was opened separately in a

clean basin of running water and the faecal pellets from the rectum were collected for further processing. With a view to obtaining some data regarding the intensity of infection with different species of Eimeria, approximately the same mass and volume of the faecal pellets were collected from each sample. Such pellets were mixed with a known volume of 2.5% Potassium dichromate solution or tap water for the purpose of determining the degree or intensity of infection in a standardised manner for all samples. As a further step in standardisation of the technique, known volumes of the faecal suspension were mixed with known volumes of saturated Sodium chloride of commercial quality and centrifuged at a fixed speed for all samples.

The oocysts in the faecal samples were allowed to sporulate in a solution of 2.5% Potassium dichromate or tap water in petri dishes of convenient size. The depth of the faecal suspension in the petri sidhes was uniformly kept at 5 mm and frequently stirred with a glass rod. The petri dishes containing different samples were incubated at 25-25°C either in the room or in a thermostatically controlled cabinet. The loss of water by evaporation from the oocyst cultures was compensated by addition of suitable quantities of fresh tap water at the same temperature as that of the cultures.

Both unsporulated and sporulated oocysts were studied in detail under the ordinary light microscope and occasionally

with the help of a phase contrast equipment. Different measurements, of as many sporulated oocysts as possible, were taken with a calibrated occulometer. The progress of the sporulation was determined at 4 hoursly intervals as far as practicable.

For setting up cross-transmission experiments, pregnant ewes were obtained and the new born lambs were maintained under strict hygienic conditions until needed. The lambs were fed bottle milk of suitable quality and stall fed with washed tree leaves and uncontaminated bran at later stages. Two such lambs, free from coccidial infections, were fed sporulated oocysts of E. faurei and E. ninakohlyakimovae obtained from goats. One lamb was fed 50,000 sporulated oocysts of E. ninakohlyakimovae in gelatin capsules. The other lamb was similarly infected with 1.6 million sporulated oocysts of E. faurei. The infected lambs were kept under close observation and the one infected with E. ninakohlyakimovae was destroyed after 16 days of infection and the gastro-intestinal tract was collected for detailed examination. The other lamb, infected with E. faurei, was kept under constant observation and relevant data of the oocyst production and their morphological features were obtained. The counting method of Stoll (1923) was utilized to compute the production of oocysts per gram of faeces and this was continued daily

till the production of oocysts per gram of faeces fell far below 100.

For studies on the various stages of Globidium species, the abomasum and intestine were thoroughly examined for macroscopic lesions. Cysts with definite contours were excised and examined in various ways. Some of them were fixed in 10% buffered formalin, Bouin's fluid and absolute alcohol and processed for preparing paraffin sections in the usual manner. Cysts suspected to be those of Globidium were also examined as squash preparations under the ordinary light and the phase contrast microscope. Cover slip preparations of the crushed cysts were also fixed and stained suitably with Geimsa stain for tissues as given by Lillie (1954), Methyl Green-Pyronin according to the method of Kurnick (1955) and 1% aquous Toludine Blue. Paraffin sections were stained with Haematoxylin and Eosin in the usual manner and with Mallory's Phosphotungstic acid Haematoxylin.

Some histochemical staining reactions were also performed on paraffin sections fixed in buffered formalin and absolute alcohol. The histochemical tests and histological stains used were :-

(i) Periodic-Acid-Schiff reaction with suitable saliva and acetylation controls,

(ii) Millon reaction for Tyrosine containing protein,

(iii) Coupled Tetrazonium reaction for proteins,

(iv) Silver impregnation for reticulum,

(v) Van Gieson for connective tissue,

(vi) Verhoeff's elastic stain,

(vii) Aldehyde Fuchsin stain for elastic tissue,

(viii) Brom phenol blue with and without Mercuric chloride for proteins,

(ix) Orcein stain for elastin,

(x) Methyl Green-Pyronin with Ribonuclease [prepared from saliva according to the method of Bradbury (1956)].

The methods for the above mentioned staining reactions were as given by Pearse (1960).

OBSERVATIONS

OBSERVATIONS

I. Survey of Eimeria in goats:

A total of 243 samples were examined and 152 faecal samples from the rectum were found positive for oocysts of Eimeria species. The majority of the infected faeces showed mixed infections with one or more types of oocysts. In all 11 species of Eimeria were encountered. The species and the number of times they were found is as follows:-

E. arloingi	105
E. parva	88
E. ninakohlyakimovae ...	81
E. faurei	48
E. hawkensi	27
E. crandallis	20
E. granulosa	15
E. pallida	9
E. ahsata	7
E. christenseni	2
E. intricata	1

The maximum number of species which was recorded in one faecal sample was 7 and these were E. arloingi, E. crandallis, E. faurei, E. granulosa, E. hawkensi, E. ninakohlyakimovae and E. parva. Six species of Eimeria in one faecal sample were encountered on two occasions. In one of them, E. arloingi, E. crandallis, E. granulosa, E. hawkensi, E. ninakohlyakimovae and E. parva were found while in the other E. ahsata, E. arloingi, E. granulosa, E. hawkensi, E. ninakohlyakimovae and E. parva were found.

Mixed infections with 5 species of Eimeria were found in 10 samples, with 4 species in 14 samples, with 3 species in 47 samples and with two species in 66 faecal samples.

The relevant data of the incidence of the different species and their intensity have been summarised in Table I.

Pure infections with single species of Eimeria were encountered on 15 occasions and of these E. arloingi was found in 5 samples, E. faurei in 2 samples; E. ninakohlyakimovae in 7 samples and E. parva in 3 faecal samples.

A perusal of Table I shows that E. arloingi, E. parva, E. ninakohlyakimovae and E. faurei are the more common species in goats of the region under survey. The incidence of the different species in goats is as follows:-

E. ahsata	4.6%
E. arloingi	69.07%
E. christenseni	1.3%
E. crandallis	13.15%
E. faurei	34.1 %
E. granulosa	9.86%
E. hawkensi	17.76%
E. intricata	0.65%
E. ninakohlyakimovae ...	53.28%
E. pallida	5.89%
E. parva	57.89%

Data regarding the seasonal incidence of Eimeria in goats have been collected and presented in Table II and Graph I. The time available for this study being very short, it has not been possible to collect data for a whole year. The data which could be gathered for 4 species during a period of seven months, however, is significant.

II. Morphology of the oocysts of Eimeria:

(A) E. ahsata: (Plate I, II)

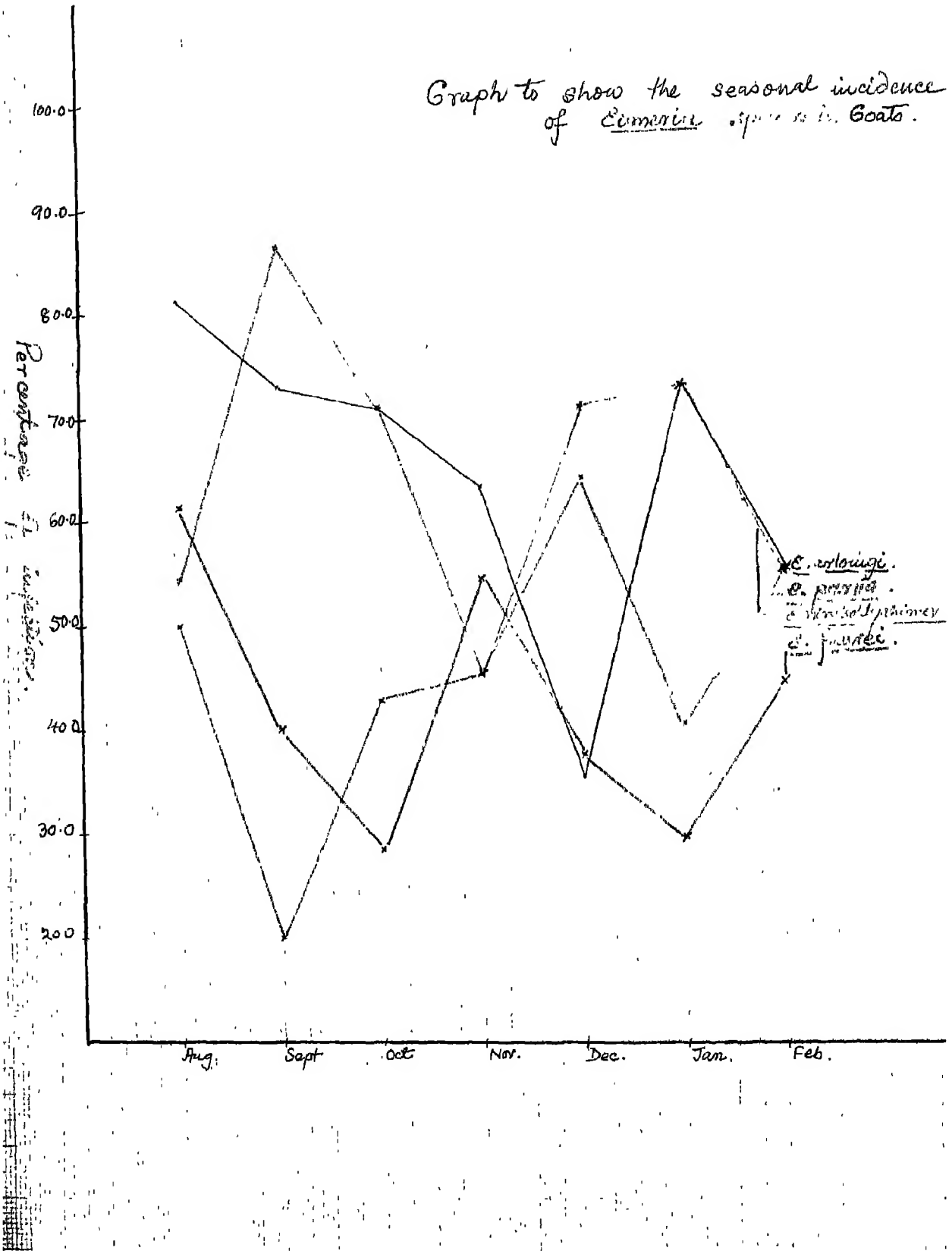
This species has not been recorded in India so far. The oocysts of E. ahsata were found on seven different

TABLE II

Table to show the seasonal incidence of *Eimeria* species in goats

Month	Percentage of infection with			
	E. arloingi	*E. parva*	*E. ninakohlyakimovae*	*E. faurei*
August 1963	81.8	54.5	50.0	61.8
September 1963	73.3	86.6	20.0	40.0
October 1963	71.4	71.4	42.8	28.5
November 1963	63.6	45.4	45.4	54.5
December 1963	35.7	64.2	71.4	35.7
January 1964	73.0	40.3	73.0	26.9
February	55.5	55.5	55.5	44.4

Graph to show the seasonal incidence of Eimeria species in Goats.



occasions and it was always mixed with other species. This species resembles E. arloingi closely and requires careful careful observation to differentiate.

The oocysts are ellipsoidal to ovoid. Both the ends are nearly rounded though at the micropylar end the inner oocyst wall appears to be somewhat flattened. The micropyle opening is not very prominent and the cap covering the opening is in the form of a shallow arch. Ten oocysts measure 38.5-40.0 x 28.0-28.8 (39.1 x 28.3) μ. The length : width ratio of the oocysts is 1.37 - 1.40 (1.38). The oocysts are pale straw coloured to light brown. The oocyst wall appears to be composed of two layers, of which the thick inner layer was more prominent and darker than the outer layer. The oocyst wall including the outer thin layer is 1.4 - 2.1 μ thick while the thick inner layer alone is 0.7-1.05 μ. The colourless micropyle caps are 2.8-3.5 μ high and 4.9-10.5 μ wide with an average of 3.1 x 7.5 μ. A few polar granules are present. The fully sporulated oocysts do not have any oocystic residuum but sporocystic residua are present (Plate II). The sporocysts are elongated ovoid and measure 11.2-14.0 x 7.0-8.4 (11.9 x 7.63) μ. A large sized refractory globule is present at the broad end of the sporozoites in addition to one or more smaller globules scattered elsewhere in the body of the sporozoites.

(B) E. arloingi: (Plate III)

This species is the most common in the goats examined. Some of the faecal samples showed heavy output of the oocysts and each field of the slide in the centrifugal floation technique showed 100-500 oocysts. A total of 503 sporulated oocysts were measured from eighteen different faecal samples.

The oocysts are mostly ellipsoidal in shape though a few of them have unequal curvature of the two sides. Such aberrant forms of the oocysts were not utilized for measurement though in all other characters they conform to the descriptions of E. arloingi. The 503 oocysts measure 21.0-41.3 x 17.5-28.0 (31.13 x 22.69) μ. On the eighteen occasions, when measurements were taken, no marked differentiation of size could be observed. The average measurements given above were invariably the same for the daily lot of measured oocysts and hence the oocysts could not be placed in one or more size groups. Very large sized oocysts were not common in each lot. For example, in the different lots used for measurements, only two or three oocysts in each lot were above 36.0 μ long.

The ratio of length : width is 1.2 - 1.47 (1.32). The thick inner layer of the oocyst wall is yellowish brown in colour while the thin outer layer is pale yellow. The oocyst wall including the thin outer layer measured 1.05 - 1.75 (1.4) μ while the prominent, thick, inner layer measures 0.35-0.70 (0.52) μ.

The micropyle and its cap is quite prominent. The colourless micropyle cap is nearly flat to hemispherical in shape and measures 1.05-3.5 μ high and 4.2 - 8.4 μ wide with a mean of 1.9 x 6.57 μ.

A few oocyst polar granules and scattered particles are present in the oocysts but oocystic residuum is absent in the sporulated oocysts. The sporocysts are ovoid to elongate ¼ in shape with the narrow and somewhat blunt and truncated. The 509 sporocysts measure 7.0 - 17.5 x 6.3 -10.5 (12.59-7.11) μ. Sporocyst residua are present. The fusiform sporozoites possess a large globule at the broad end and a smaller one at the other extremity.

(C) E. christenseni: (Plate IV; Fig. 1)

The oocysts of E. christenseni were located on two occasions in adult goats and this becomes the first record for India and anywhere else other than United States of America, where it was first described (Levine, Ivens & Fritz, 1962).

The oocysts are oval to ovoid in shape with the micropylar end being slightly narrower and somewhat flattened. Six oocysts measure 33.6 - 43.4 x 24.5-28.0 (39.43-26.83) μ and the length : width ratio is 1.37-1.55 (1.47). The oocyst wall is yellowish brown to cherry brown in colour and measures 1.75 - 2.10 μ in thickness with the inner prominent wall being 0.7-1.05 μ.

The narrow end of the oocyst has a distinct micropyle and its colourless, hemispherical cap measures 1.05-2.45 (1.75) μ high and 7.00-8.40 (7.35) μ in width.

The fourteen sporocysts measure 11.2-15.4 x 8.4-10.5 (13.3 x 8.9) μ. The sporulated oocysts do not have any residuum but sporocystic residua are present.

(D) E. crandallis: (Plate V)

The oocysts of E. crandallis were not very common. The oocysts are ellipsoidal to ovoid. The micropylar end is flattened. The micropyle is not distinct. Eight oocysts measure 21.0 - 24.0 x 16.6-19.6 (22.4 x 18.2) μ. The oocyst is colourless to pale yellow in colour. The oocyst walls are formed by two layers of which, the inner thick wall is pale yellow in colour while the outer is colourless. The colourless micropylar caps are flattened and measure 0.7-3.5 μ high and 4.2-8.7 μ wide with an average of 2.1 x 5.6 μ. In the fully sporulated oocysts the oocystic residuum is absent but sporocystic residua are present. The sixteen sporocysts are ovoid and measure 6.3-10.5 x 5.6-7.0 (9.6-6.0) μ.

(E) E. faurei (Plate VI)

The oocysts are egg shaped. The micropylar end is pointed and conical. The 492 oocysts from eleven faecal samples measure 23.8-37.8 x 18.2-29.4 (30.55 x 22.30) μ.

The length : width ratio of the oocysts is 1.30-1.47 (1.37) The oocyst wall apprears to be composed of two layers of which the inner thick layer has a bluish or yellowish tinge and the outer thin layer is colourless. The total thickness of the two layers is 1.05-2.1 μ while the inner thick wall alone measures 0.7-1.05 μ. The micropyle is distinct. There is no cap over the micropyle. The polar granules are absent. The fully sporulated oocyst does not have either the oocystic residuum or the sporocystic residua. The 495 sporocysts are elongate ovoid and measure 7.7-15.4 x 6.3-10.5 (11.92 x 7.05) μ.

(F) E. granulosa (Plate VII)

A total of 183 sporulated oocysts were measured from 3 different faecal samples. The oocysts are pyriform in shape. The micropylar end of the oocysts is more prominent and broader than the other end. The 183 oocysts measure 28.7-38.5 x 21.0-28.0 (32.25 x 23.72) μ. The length : width ratio is 1.37-1.40 (1.36). The oocyst wall is composed of two distinct layers. The inner layer of the oocyst wall is yellowish brown in colour and alone measures 0.7-1.05 μ thick. The outer thin layer of the oocyst wall is pale straw coloured. The total thickness of the oocyst wall including both the layers is 1.05-2.10 μ. There is a distinct micropyle covered by a colourless flattened cap, which is 2.1-2.8 μ high and 4.9-7.7 μ wide with an average of 2.45 x 6.65 μ. There are no polar granules. The oocystic residuum is absent in the fully sporulated oocysts

but sporocystic residua are present. The sporocysts are ovoid and measure 8.4-12.6 x 6.3-8.4 (10.5-7.0) μ. A large sized refractory globule is present on both the ends of the sporozoites.

(G) E. intricata: (Plate VIII)

This species has been found only once in the goats examined. The oocysts are ellipsoidal in shape and are yellowish brown to dark brown in colour. The 75 oocysts measure 44.1-53.2 x 37.5-41.3 (50.42 x 38.85) μ. The ratio of length : width is 1.15-1.30 (1.23). The oocyst wall appears to be made up of 3 layers. The outermost is transparent and difficult to discern. The middle layer is brown to dark brown in colour and is the thickest of all. The thickness of the middle layer appears to be transversely striated. The innermost oocyst wall is thin and colourless. The total thickness of the oocyst wall including all the three layers is 2.1-3.5 μ while that of the middle layer alone is 1.05-1.75 μ. The micropyle is distinct and covered by the micropyle cap, which is colourless to yellowish green in colour. The cap is semi-lunar in shape or boat-shaped. Quite often the top of the concavity is somewhat flattened and the edges of this area are raised or notched. The caps measure 1.4-5.6 μ in height and 7.0-16.0 μ in width with an average of 2.74 x 13.73 μ. The polar granules and oocystic

residua are absent, but sporocystic residua are present. The embryonic mass in the unsporulated oocysts measuring 25.2-28.0 μ in diameter, are mostly located towards the micropylar end of the oocysts and seldom in the opposite end. The sporocysts are elongated ovoid with prominent steidae bodies and measure 13.3-19.6 x 8.4-11.2 (17.7 x 9.94) μ. A large number of refractile globules are present in the sporozoites.

(I) E. hawkensi: (Fig. 2)

This species requires careful observation to locate since the important point for identification is the nature of the triangular cap on the oocysts. The oocysts are ovoid to ellipsoidal in shape and pale pinkish to yellowish green in colour. The measurement of 50 sporulated oocysts were taken from 5 different lots of faecal samples and the same is 19.6-30.1 x 16.8-21.0 (26.62 x 18.36) μ. Their length : width ratio is 1.16-1.43 (1.3). The oocyst wall appears to be made up of two layers. The outer layer is a thin colourless membrane surrounding the inner thick wall. The total thickness of the oocyst wall is 1.05-1.40 μ, while the inner prominent wall is 0.35-0.7 μ. The micropyle is not easily discernible. The triangular micropylar cap is 0.7-2.8 μ high and 4.9-7.7 μ in width, with an average of 1.36 x 6.27 μ. The sporocysts are ovoid in shape and measure 7.0x12.6 x 4.2-7.7 (9.91 x 6.44) μ.

Polar granules and oocystic residuum is absent but sporocystic residua are present.

(I) E. ninakohlyakimovae (Plate IX)

The oocysts were studied from 7 different faecal samples. The oocysts are sub-spherical to ellipsoidal. The micropylar end is marked off by a prominent darker coloured ridge. A total of 505 oocysts were studied and they measure 18.2-28.0 x 16.8-21.0 (23.97 x 19.0) μ. The oocysts fall into two categories of size with the ratio of the larger to the smaller forms being 4:1. The ratio of length : width is 1.04-1.33 (1.23) μ. The oocyst wall is made up of two layers. The inner thick layer is faint bluish in colour and measures 0.7-1.05 μ. The outer wall is thin and colourless. The total thickness of oocyst wall including the thin outer layer is 1.05-1.40 μ. The micropyle is indistinct and the micropylar cap is absent. There are no polar granules. The fully sporulated oocyst does not have any oocystic residuum but sporycstic residua are present. The sporocysts are ovoid in shape and measure 4.2-13.3 x 4.2-7.0 (9.17 x 6.27) μ.

(J) E. pallida: (Plate X)

The oocysts are pale yellow to greyish yellow in colour and sub-spherical to ellipsoidal in shape. The 10 oocysts which were measured are 14.2-15.4 x 11.2-12.6 μ (14.5x11.9) μ.

The oocyst wall is composed of 2 layers, the outer wall which is not distinct and the inner thick wall, which is pale yellowish in colour and measures 0.35-0.70 μ in thickness. The total thickness of the wall including the outer layer is 1.05-1.40 μ. There is neither a micropylar nor a polar cap. Oocyst polar granules and oocyst residuum are absent but sporocystic residua are present. The sporocysts measure 4.5-9.8 x 3.8-5.6 (7.06 x 4.7) μ. The validity of these forms is not very clear. This is further discussed later on.

(K) E. parva : (Plate XI)

The oocysts are spherical in shape. The 72 oocysts from 3 different faecal samples, which were studied, measure 11.2-26.4 x 9.8-21.0 (18.06 x 16.02) μ. Their length : width ratio is 1.14-1.36 (1.12). The oocyst wall is refractile, smooth and faint yellow to pale yellowish green in colour. The total thickness of the oocyst wall is 1.05-1.40 μ and the thick inner layer measures 0.35-0.70 μ in thickness. The outer layer of the oocyst wall is thin, membranous and colourless. There is neither a micropylar nor a cap. There are also no polar granules, oocystic and sporocystic residua. The sporocysts are ovoid and measure 4.2-6.3 x 4.2-5.6 (5.6 x 4.9) μ.

The relevant data of the various species of Eimeria have been summarised in Table III.

TABLE III

Table showing morphology og oocysts

Note: All measurements are in microns

Sl. No.	Species	Total No. of oocysts measured	Average size of oocysts	Average size of Micropyle cap	Average size of sporocysts	Thickness of cyst wall
1.	E. ahsata	10	39.1 x 28.3	7.5 x 3.1	11.9 x 7.0	1.4 - 2.1
2.	E. arloingi	303	31.13 x 22.69	6.57 x 1.91	12.59 x 7.11	1.05-1.75
3.	E. christenseni	6	39.43 x 26.83	7.35 x 1.75	13.3 x 8.9	1.75-2.10
4.	E. crandallis	8	22.4 x 18.2	5.6 x 2.1	9.6 x 6.0	-
5.	E. faurei	492	30.55 x 22.30	-	11.92 x 7.05	1.05-2.1
6.	E. granulosa	183	32.25 x 23.72	6.65 x 2.45	10.5 x 7.0	1.05-2.1
7.	E. intricata	75	50.42 x 38.85	13.76 x 2.76	17.74 x 9.94	2.1 -3.5
8.	E. hawkensi	50	26.62 x 19.36	6.27 x 1.36	9.91 x 1.05	0.7 -1.05
9.	E. ninakohlyakimovae	505	23.97 x 19.0	-	9.17 x 6.27	1.05-1.40
10.	E. pallida	10	14.5 x 11.9	-	7.06 x 4.70	-
11.	E. parva	72	18.06 x 16.02	-	5.6 x 4.9	1.05-1.40

III. Sporulation of the oocysts:

The progress of sporulation of the different oocysts has been watched under a constant temperature of 25° ± 1°C in a thermostatically controlled cabinet and under room temperature condition which shows diurnal variation. The details of sporulation are given below and have also been summarised in Table IV.

A. E. ahsata:- The sporoblastic stage is reached in 32 hours at 25°C while the sporocystic stage requires another 24 hours. The oocysts are fully sporulated in 72 hours. At 17-19°C the sporulation is delayed and the same requires 90 hours for completion.

B. E. arloingi:- The sporozoites are fully formed in 48 hours at 25°C. The sporoblastic stage is reached within 24 hours and the sporocysts are differentiated in another 12 hours. At 28°-32°C the sporulation of the oocysts is not hastened.

C. E. crandallis:- The sporulation time is the same as E. arloingi, i.e., 48 hours at 25°C.

D. E. faurei:- Under room temperature of 23° - 30°C the oocyst requires 72 hours for the sporoblasts to be formed and then another twelve hours each for the sporocysts and sporozoites to be formed, respectively. At 26°C, however, the sporulation is complete in 72 hours.

E. E. granulosa:- The oocysts are fully sporulated in 72 hours at 26°C. The sporoblasts are formed in 56 hours and

the sporocysts in another 12 hours. Under room temperature conditions of 23-30°C the time taken for sporulation is somewhat variable from 72-96 hours.

F. E. hawkensi:- At 25°C the sporoblastic stage is reached in 32 hours, the sporocystic stage in another 8 hours and the sporozoites are fully formed in 48 hours from the commence. At 17-19°C the oocysts are sporulated in 64 hours.

G. E. intricata:- The oocysts are fully sporulated in 7 days at 25°C. The sporoblasts are formed in 72 hours, while the sporocysts require another 48 hours to form. Under room temperature conditions the time taken for sporulation varies from 5-8 days.

H. E. ninakohlyakimovae:- At 26°C the sporulation of oocyst commences in 36 hours with the sporoblastic stage being discernible and the same is completed to the sporozoitic stage in 56 hours. At 24-30°C the time for sporulation is prolonged to 72 hours.

I. E. pallida:- The oocysts require 24-36 hours at 26°C for complete sporulation. At 18-22°C the time for sporulation is prolonged to 48 hours.

J. E. parva:- At 25°C the oocysts become fully sporulated in 48-72 hours, while at 29-32°C the time taken is 24-48 hours.

K. E. christenseni:- At 26°C sporoblasts are formed in 48 hours and sporozoit stage is reached in 72 hours.

TABLE IV

Table to show sporulation of Eimeria oocysts

Sl. No.	Species	TIME REQUIRED							
		At room temperature				At 25° - 26°C			
		Sporoblastic stage Hrs.	Sporocystic stage Hrs.	Sporozoite stage Hrs.	Total Time Hrs.	Sporoblastic stage Hrs.	Sporocystic stage Hrs.	Sporozoite stage Hrs.	Total Time Hrs.
1.	E. ahsata	48	72	90	48 - 90	32	56	72	32 - 72
2.	E. arloingi	24	36	48	24 - 48	24	36	48	24 - 48
3.	E. crandallis	24	36	48	24 - 48	24	36	48	24 - 48
4.	E. faurei	72	84	96	72 - 96	48	56	72	48 - 72
5.	E. granulosa	72	84	96	72 - 96	56	64	72	56 - 72
6.	E. hawkensi	50	56	64	50 - 64	32	40	48	32 - 48
7.	E. intricata	120	160	192	120 -192	72	120	168	72 -168
8.	E. ninakohlyakimovae	56	64	72	56 - 72	36	44	56	36 - 56
9.	E. pallida	24	-	46	24 - 48	24	-	36	24 - 36
10.	E. parva	48	-	72	48 - 72	24	-	48	24 - 48
11.	E. christenseni	-	-	-	-	48	-	72	48 - 72

IV. Cross-transmission:

(a) E. faurei from goat to lamb: It has been possible to successfully cross-transmit E. faurei from goat to sheep. A goat having a pure infection of E. faurei was used as the donor animal. One lamb, aged 40 days and free of any parasitic infection, was fed with 1.6 million sporulated oocysts of E. faurei. The oocysts were fed with the help of gelatin capsules. Subsequently, the faeces of the infected lamb was collected in faecal bags and examined for the presence of oocysts. Twelve days after infection the faeces revealed the presence of oocysts, which on examination was found to be that of E. faurei. The oocyst per gram of faeces was determined daily and the relevant data has been given in Table V.

TABLE V

Table to show the daily output of oocysts of E. faurei per gram of faeces

Date	Oocysts per gram of faeces
26-1-64	21000
27-1-64	330,00
28-1-64	680,00
29-1-64	390,00
30-1-64	310,00
31-1-64	250,00
1-2-64	230,00
2-2-64	219,00
3-2-64	190,00
4-2-64	168,00
5-2-64	135,00
6-2-64	82,00
7-2-64	210,00
8-2-64	145,00
9-2-64	91,00

Date	Oocysts per gram of faeces
10-2-64	23,00
11-2-64	13,00
12-2-64	7,00
13-2-64	Less than 100
14-2-64	-do-
15-2-64	-do-
16-2-64	-do-

On the day of infection, the lamb weighed 3.85 kg. and after 35 days, when the observations were discontinued the weight increased to 6.8 kg. Throughout this period, the lamb remained healthy and showed no clinical signs of coccidiosis. The prepatent period was thirteen days, when oocysts began to appear in the faeces. The production of oocysts continued for 22 days when the quantum of output became very low.

The oocysts are similar to the descriptions given for E. faurei. A total of 50 oocysts measure 24.3-36.7 x 19.6-28.7 (30.0 x 21.6) μ. The sporocysts measure 8.4-14.0 x 6.3-9.8 (11.2 x 7.0) μ. These oocysts undergo complete sporulation in 48-72 hours at 25°C.

(b) E. ninakohlyakimovae from goat to lamb:

One goat showing a pure infection of E. ninakohlyakimovae was used as a donor animal. A lamb, aged 16 days, weighing 2.95 kg. and free from any infection was fed 50,000 sporulated oocysts of E. ninakohlyakimovae in gelatin capsules.

On the 11th day of infection, oocysts of E. ninakohlyakimovae began to appear in the faeces. The daily output

of oocysts per gram of faeces has been determined and the data has been summarised in Table VI.

TABLE VI

Table showing the output of oocysts of *E. ninakohlyakimovae* in the infected lamb

Date			OOcysts per gm. of faeces
30-1-64	...	...	170,000
31-1-64	...	...	300,000
1-2-64	...	...	435,000
2-2-64	...	...	265,000
3-2-64	...	...	203,000
Slaughtered on 4-2-1964			

The lamb showed clinical manifestations of coccidiosis on the 11th day and onwards. The signs were mucus in faeces, imperfectly formed faecal pellets and general deterioration of condition. On the 14th day the faeces was diarrhoeic. The lamb was slaughtered on the 15th day of infection.

On post-mortem examination echymosis and haemorrhagic tracts were noticeable through the whole length of the small intestine. There were marked petechial lesions in the mucosa of the small intestine, specially the ileum and colon. Areas of denuded of the epithelium were also noticed.

Microscopial examination revealed desquamation of the epithelial lining of the villi in the small intestine. The

epithelial cells contained various developmental stages of E. ninakohlyakimovae (Plates XII, XIII and XIV). The schizonts are present in the same region of the intestine as the gaemetocytes. The schizonts are 16-30 μ in diameter and contained 100 and odd merozoites. The ileum and colon show the infected areas as thickened and oedematous lesions with extensive leucocytic infiltration and hyperplasia of the epithelium.

The oocysts have the same morphology as in a natural infection of E. ninakohlyakimovae in goats. A total of 50 oocysts from the experimentally infected lambs measured 19.6-27.3 x 17.5-20.3 (23.3 x 18.57) μ. The sporocysts in the sporulated oocysts measure 4.9-12.6 x 5.6-6.3 (8.3 x 5.8) μ.

V. Survey of Globidium gilruthi in goats:

58.44% of the gastro-intestinal tracts examined were found positive for the presence of Globidial cysts. The cysts of Globidium gilruthi were found in the abomasum, duodenum, jejunum and ileum. In the abomasum, the cysts were encountered on 35 different occasions, in the duodenum 95 times, in the jejunum on 79 occasions and in the ileum in 44 samples.

VI. Morphology and histochemistry of the Globidium cysts: (Plates XV to XXX)

The cysts in the abomasum are deeply embedded in the submucosa. Those in the small intestine are more superficial, most of them being in the submucosa and some in the mucosa.

The cysts in the abomasum are mostly in the region of the cardiac end. Most of the cysts are round, pin-head sized bodies and yellowish-white in colour. Some of the cysts are reddish in colour and this is evidently due to haemorrhage in the adjoining tissue. The unfixed cysts in the abomasum, in situ, measure 0.5-2 mm. in diameter.

The abomasum has few to many such cysts and in one case, when actual count was taken, there were as many as 372 cysts.

The cysts in the duodenum and jejunum are smaller in size and more superficial in position. With the naked eye these cysts are pin point size bodies and slightly raised from the general surface of the epithelium.

In the abomasum and the intestine some of the cysts had burst and released their contents as is evident by the punched hole appearance of some of them. In sectioned material some of the cysts could be seen in the ruptured condition with the spores liberated into the disorganised epithelial layer (Plate XXIII). The cysts in the paraffin sections of the abomasum were measured and these showed a lot of variations. The round to oval sections of the cysts are of two sizes. The larger sized cysts measure 103.5-801.0 x 69.0-572.0 (455.7 x 356.7) μ. The smaller sized cysts measure 136.0-586.5 x 120-0 - 414.0 (399.3 x 304.45) μ. Generally the cysts fall into two size groups which is also correlated with differences in their internal morphology.

This aspect has been further described below.

The cysts are generally of two types. One of them, which is somewhat smaller than the other has a definite cyst wall and contains large sized spores or merozoites. This is the schizont stage. The other type also has a cyst wall but the same is comparatively thinner and the contents comprise of humerous small spore like structures. This is the microgametocyte. The nuclei of the "spores" or merozoites in a schizont are much larger in size than those of the microgametes in a microgametocyte. The "spores" or merozoites are boat to comma shaped, measure 6.3-9.1 x 1.75-2.80 (7.3 x 2.2) μ and have a prominent globule in the middle of the body and over the nucleus. Under the phase contrast microscope, the "spores" or merozoites appear in the form of a sickel shaped body with one end blunt and rounded and the other tapering and with a central prominence indicating the nucleus and the globidial body. The cyst wall in the schizont measures 2.8-5.6 μ in thickness, while those of the microgametocyte measures 1.05-3.5 μ.

The schizonts, which have been studied, are mature or nearly mature (Plates XVI, XVII, XVIII, XIX). In many cases the merozoites could be seen in different stages of formation. One of the stages is the blastophore stage (Plate XVII). In a schizont the blastophores are present in the form of round to oval bodies with the nuclei lying peripherally in a single row (Plates XVI, XVII). The central area in a blastophore

is vacuolated and devoid of nuclear material (Plate XVII). The merozoites are budded off from the peripheral surface of the blastophores.

The cyst wall of the schizont is well formed and prominent. In a few paraffin sections, the thickness of the cyst wall has numerous vacuolated areas (Plates XVI, XVII). In some, the cyst wall has a fuzzy or furry outgrowth. In all probability these are artefacts of fixation as such a character is not found in every case. It is more common to find the cyst enclosed by a thick wall which is homogenous and not striated or laminated prominently.

The cyst wall of the schizont is very weakly Periodic Acid-Schiff (PAS) positive, saliva fast, and negative to PAS reaction after accetylation; orthochromatic with thiazine dyes; positive for the millon reaction, Brom phenol blue and coupled tetrazonium reaction; pyrininophilic in the Methyl Green-Pyronin technique; stains with aldehyde fuchsin stain, Verhoeff's stain and Orcein stain; does not stain pink or red with Van Gieson's connective tissue stain; is very weakly argentophilic in the silver impregnation technique; stains rose red to pink with Mallory's phosphotungstic acid haematoxylin; and is eosinophilic in the haematoxylin and eosin sequence. The above results of the histochemical staining reactions indicate elastin or elastic type of tissue in the cyst wall of the schizonts. A host cell or its remnants is

not evident around the schizonts. There is, however, extensive infiltration of the surrounding tissue with leucocytes and macrophages (Plates XX, XXVII, XXIX, XXX).

The globidial bodies in the merozoites are transparent and eosinophilic. Histochemically, these are weakly PAS-positive, poorly pyrininophilic and positive for the millon reaction, coupled tetrazonium reaction and stains with Brom phenol blue in the presence of mercuric chloride.

The microgametocytes are larger than the schizonts. The contents of a mature microgametocyte comprise of numerous microgametes without any residual bodies as in the schizonts. Globidial bodies are not present in the microgametes. The microgametes are also formed through the intermediate stage of blastophores, which, however, are much smaller and irregular in size than those of the schizonts.

Besides the schizont and microgametocyte, no other stages in the life-cycle of Globidium gilruthi could be encountered. No macrogametocytes, macrogametes or oocysts were seen. One oocyst, which was observed in a faecal sample from the rectum is unlike the oocysts of Eimeria species. The oocyst is obovate in shape and measures 53.0 x 37.5 μ. The wall of the oocyst is very thick and no outer thin layer is discernible. The oocyst wall measures 6-8 μ. The micropyle is present but there is no micropyle cap. It is difficult to say if this oocyst could be considered to belong to Globidium gilruthi.

The cysts in the duodenum are 0.3 to 1 mm in size, yellowish white in colour and are of two types. The larger microgametocytes in section, measure 207.0-345.0 μ in diameter, while the schizont, which are smaller in size, measure 178.0-276.0 μ in diameter. The cysts in the jejunum and ileum are also of two types. The schizonts measure 138.0-345.0 x 110.4-345.0 (282.9 x 215.2) μ. The microgametocytes measure 241.5-345.0 x 172.5-241.5 (286.25 x 210.25) μ.

DISCUSSION

DISCUSSION

Earlier workers in India (Ray, 1949; Sharma, 1951-52; Rao and Hiregaudar, 1953-54 and Manjrekar, 1954) have recorded the incidence of Eimeria species in sheep and goats. Except for Sharma (1951-52), all the other workers have indicated the occurrence of Eimeria species in both the caprine and ovine hosts. During the course of the present survey eleven species of Eimeria were recorded from goats and of these, two species, E. ahsata and E. christenseni, have been recorded for the first time in this country.

In order of importance and high incidence, E. arloingi is the most important species in goats followed by E. parva, E. ninakohlyakimovae and E. faurei. The incidence of the other species was not so high as in the above mentioned four species.

It was uncommon to find pure infections of simple species of Eimeria in the goats. Quite often, more than one species was encountered in the same goat. As many as seven species, viz. E. arloingi, E. crandallis, E. faurei, E. granulosa, E. hawkensi, E. ninakohlyakimovae and E. parva, were found in a single host on more than one occasion. Mixed infections with 5-3 species were quite common.

Detailed records of the seasonal incidence of the various species of Eimeria in the goats were not obtained. It was, however, evident that humid and low temperature conditions favour a higher intensity of infection. E. arloingi,

E. parva, E. ninakohlyakimovae and E. faurei have their maximum incidence from October to December.

Honess (1942) recorded E. ahsata (= E. ah-sa-ta) in the Rocky Mountain big-horn sheep and subsequently, this species has also been recorded in the lambs of America (Smith, Davis & Bowman, 1960; Smith & Davis, 1961 and Levine, Ivens, Smith & Davis, 1962). This species has now been found in the goats during the course of the present survey. E. ahsata was found on eight different occasions intermixed with other species. This species has some resemblance to E. arloingi, E. christenseni and E. crandallis. In its average size, E. ahsata is slightly bigger than E. arloingi though in the characters of micropyle cap, oocystic and sporocystic residua, they resemble each other. The size of the oocysts of E. ahsata, recovered in the goat faeces, ranges from 38.5 - 40.0 x 28.0 - 28.8 (39.1 x 28.3) μ. This range of size variation does not come close to the measurement given by Honess (1942), but comes within the range given by Levine, et al. (1962). E. ahsata differs from E. christenseni in the shape of the oocyst and sporocyst and the length width ratio. The differences between E. crandallis and E. ahsata are in the size of the oocyst and sporocyst and the nature of the micropyle cap.

The dimensions of the oocysts of E. arloingi in goats has been provided by many workers (Table VII). The

measurement of the various structures in the oocysts have not been provided by all workers. A fairly large number of oocysts (503 oocysts) of E. arloingi have been studied and measured. A perusal of Table VII shows that the measurements fall within the limits obtained by other workers, though the range of variation is much greater. The oocysts are not graded into one or more size groups.

Levine et al. (1962) brought forth evidence to show that the upper range in size of the oocysts of E. arloingi given by Christensen (1938) probably included oocysts of E. ahsata. During the present study a range of size (21.0-41.3 x 17.5-28.0 (31.1 x 22.6)μ) similar to that given by Christensen (1938) was encountered. Large sized oocysts were invariably observed closely and it was concluded that none of them belonged to E. ahsata. It is thus evident that a lot of size variation is possible in the oocysts of E. arloingi.

The polar cap in the oocysts of E. arloingi has been variously described as half moon-shaped, helmet-shaped catapult-shaped or flat to hemispherical in shape. It has also been recorded that the caps are easily dislodged specially when kept for long duration in salt solution. It was, however, found that the caps are not easily dislodged and lost and that these can remain intact and in situ even when subjected to strong osmotic pressure in saturated salt solution for 2-6 hours.

Yakimoff (1931) had described a new species, E. aemula, but Christensen (1938), Hardcastle (1943) and Pellerdy (1956) synonymised it with E. arloingi. This was done because the specific characters are variable and specially for the fact that oocysts ofE. arloingi, which have lost the cap, could easily be confused for the alleged oocysts of E. aemula. It is, however, likely that the synonymy of E. aemula lies with E. faurei rather than E. arloingi (Levine, 1961).

Melikian (1955) said that E. arloingi is not a valid species and synonymised it with E. faurei. This new point has not been accepted by most protozoologists and E. arloingi and E. faurei are both to be regarded as valid species and infecting both the ovine and caprine hosts (Lotze, 1953; Rysavy, 1954; Svanbaev, 1957; Krilov, 1961; Levine et al., 1962).

Krilov (1961) believed that the forms of E. arloingi in sheep and the goats are xenodemes. Krilov's observation, however, is open to criticism, in as much as, the acquired immunity of the experimental animals under his observation, does not appear to have been determined.

The oocysts of E. christenseni have some resemblance with that of E. ahsata. The oocyst of E. crandallis has a size variation which falls near the small sized oocysts of E. arloingi and the oocysts of E. parva and E. ninakohlyakimovae. The micropyle cap in E. crandallis is not well developed and hence liable to be missed. The presence of the cap, however,

is an important character by which this species could be differentiated from E. parva and E. ninakohlyakimovae besides the shape of the oocyst. Lotze (1953) doubted the validity of E. crandallis but Levine et al. (1962) have established its individuality. During the course of the present work, E. crandallis had an incidence of 13.15% and was invariably found mixed with other species.

E. faurei is quite common with 34.1% infection. In its morphology and biological features, the description tallies with those available in the literature.

Christensen (1938) described E. granulosa for the first time from sheep but the description was incomplete in certain respects. The average size of the oocysts available during the present survey are somewhat bigger than the measurements given by Christensen (1938). The sporulated oocysts have sporocystic residua but these were not described by Christensen (1938).

E. hawkensi was recorded for the first time in India (Ray, 1952) but strangely enough this has neither been reviewed nor accepted as a valid species by workers outside the country. In all probability the inaccesibility of the only published account (abstract in the Proceedings of the Indian Science Conference) has been responsible for this state of affairs.

Ray (1952) has given an important feature in the oocysts of E. hawkensi by which it could be differentiated

from the other known species. The polar cap is triangular in shape and originates from the endocystic wall. In size, E. hawkensi in the present collection appears somewhat bigger than that of Ray (1952). The sporulated oocysts have sporocystic residua but oocystic residuum is absent. The sporocysts are ovoid to ellipsoidal. The sporulation of the oocysts is complete in 24 hours at room temperature and prolonged immersion in saturated salt solution damages the polar cap causing artefacts and making it difficult to identify.

The oocysts of E. intricata was encountered only once in an adult goat. The morphology of the oocysts agrees closely with the description given by Christensen (1938). An oocystic residuum is absent but sporocystic residua are present.

E. ninakohlyakimovae is quite common in the goats examined and the incidence is 53.28%. The oocysts of E. ninakohlyakimovae fall into two groups, round and oval, and these are present in the ratio of 4:1. The round forms were originally placed in another species, viz., E. galouzi by Yakimoff & Restegaeiff (1930) but subsequent workers (Balozet, 1932; Becker, 1934; Christensen, 1938) consider these as variants of the oval form of E. ninakohlyakimovae. The examination of the present material shows that there is a gradation from the round to the oval forms. When such oocysts are fed to an experimental lamb, the resulting

crop of oocysts again show the same range of size variation.

The validity of E. pallida is not very clear. Pellerdy (1956) synonymized it with E. parva but Levine (1961) and Richardson & Kendall (1963) have considered it as a valid species. There are not many important points of difference between E. pallida and E. parva. From the biometrical data available in the present study, the oocysts of E. parva are somewhat bigger than those of E. pallida. The only point of important difference between the two is the pale to colourless, fragile wall of E. pallida in contrast to the stronger, yellowish oocyst wall of E. parva, besides the indistinct sporocystic residuum in E. parva and its absence in E. pallida. In the present material, however, sporocystic residua appear to be present in E. pallida but absent in E. parva. Thus, the characters of E. pallida and E. parva are not well differentiated.

Colour of the oocystic wall is a character which could show imperceptible variations and which is also subject to errors of vision. The original description of E. pallida by Christensen (1938) does not mention about the presence or absence of sporocystic residua. Christensen (1938) had based his specific diagnosis on the colour and fragility of the oocyst wall. Since Levine (1961) describes the presence of indistinct sporocystic residua occasionally in E. parva and since the present material suspected to be E. pallida, has this feature, there is thus a possibility that E. pallida and E. parva are synonyms.

The sporulation of the oocysts of the various species have been determined under uniform controlled conditions (Table IV). Sporulation time for E. ahsata, E. christenseni, and E. crandallis have been obtained as the same are not available in the published literature.

It has generally been assumed by many workers that Eimeria species are common to sheep and goats. Experimental evidence, however, is not available in all cases. Baloset (1932c) had failed to transmit E. ninakohlyakimovae from ovine to caprine host. Krilov (1961) failed to transmit E. arloingi from sheep to goat and vice versa. Lotze (1957) believes that it is only an assumption that all the ten species of Eimeria recorded in U.S.A. would affect both sheep and goats.

The forms of Eimeria found in sheep and goats are morphologically similar and it remains to be shown if these are different biological strains or races. Krilov (1961) thought that E. arloingi in sheep is a different race. Krilov's (1961) failure to cross transmit E. arloingi from sheep to caprine host is subject to the criticism that the caprine host used for demonstrating the biological races in E. arloingi was actually one which presumably had already picked up a similar infection in nature and hence developed resistance or immunity to reinfection.

During the course of the present work it has been possible to transmit successfully E. faurei and E. ninakohlyakimovae from goat to young lambs. The infection of E. faurei

and E. ninakohlyakimovae in the experimental lambs was clear cut and normal. The life cycle of E. faurei is not fully known. The prepatent period in the experimental lamb is 12 days. The maximum output of oocysts is reached on the 15th day and then is a steady decline upto the 22nd day or so. Subsequently, the output again goes up, reaching its peak on the 25th day (Table IV). Then again the production of oocysts begins to fall and by the 32nd day, the output is negligible.

The maximum output of the oocysts on the 15th and 25th days presumably corresponds to the formation of the first generation and second generation schizonts. The formation of the first generation schizont and the subsequent gametocyte stages is completed by the 15th day when maximum number of oocysts are voided out. The formation of the second generation schizont and the succeeding sexual stages presumably require another 8-10 days as is evident by the increased output of oocysts on the 25th day of infection. The production of oocysts declines after the 25th day and falls off to a negligible level. The self limited life cycle is thus completed in about 32 days in the absence of reinfection and there are only two generations of schizonts.

The production of oocysts and their morphological features are as normal as in a natural infection in goats. The age of the experimental lamb is such that it does not throw any light on the factor of age resistance. The use of a

very young animal free from prior natural infection has evidently helped in establishing cross-transmission of E. faurei from goat to sheep.

The life cycle of E. ninakohlyakimovae has been described by Balozet (1932b) and Lotze (1954) in goat and sheep respectively. The prepatent period is 15 days in lamb (Shumard, 1957) and 10-13 days in goat (Balozet, 1932b). In the present experiment, when oocysts of E. ninakohlyakimovae obtained from a goat were fed to a young lamb, the oocysts began to appear in the faeces on the 11th day. This shows the prepatent period to be 10 days and thus agrees with Balozet's (1932b) observation in goat. Hence the prepatent period appears to be the same in both sheep and goat.

The output of oocysts, which commences on the 11th day of infection, reaches its maximum on the 13th day of infection and then it begins to fall.

The production of oocysts and their morphology in the experimental lamb is normal and a clear cut infection is established. This shows that E. ninakohlyakimovae, which exists in morphologically similar forms in both caprine and ovine hosts, is transmissible from goat to sheep.

The validity of the genus Globidium is uncertain. Of late, there is a tendency to relegate this parasite to the synonymy of Eimeria (Reichenow & Carini, 1937; Reichenow, 1940, 1953; Pellerdy, 1960). Pellerdy (1956, 1960) believes that the taxonomic status of Globidium must be sought among the Eimeria species and the classification of the latter should be suitably amended.

According to Pellerdy (1960) the amended generic diagnosis given by Henry & Masson (1932) is dubious and of no real value. The large type of oocyst associated with Globidium resembles closely the corresponding stage in different Eimeria species, viz., E. intricata in sheep and goats; E. ponderosa Wetzel, 1942 and E. superba Pellerdy, 1955 in roe deer; E. scabra Henry 1931 in pig and E. sculpta Madsen, 1938 in hare. Similarly, the Globidial type of schizont has been found in E. bovis (Hammond et al., 1944, 1946), in E. parva (Kotlan, Pellerdy & Versenyi, 1951) and in E. seideli (Pellerdy, 1960).

There are, however, certain characteristics in the Globidial type of cysts which are not found in Eimeria species. The Globidium type of cyst has a definite cyst wall and the cysts are invariably embedded deep in the mucosa. The 'spores' or merozoites in the Globidium type of cysts are large and have inclusions in the form of refractile globidium bodies. In the case of G. gilruthi at least, the schizont and microgaemetocyte type of cysts are found in the abomasum also while none of the stages of Eimeria have been found in this region of the gastrointestinal tract. There are thus certain characters which give some degree of individuality to the Globidium type of cysts.

The histochemistry of the cyst wall in G. gilruthi reveals that it has a thick wall made up of elastin or elastic type of tissue. It is quite possible that a similar

wall is demonstrable in the Eimeria type of schizonts. This, however, remains to be shown histochemically and histologically.

The "spores" or merozoites in a G. gilruthi cyst are usually large and innumerable in number. If all of them are to be regarded as merozoites of Eimeria then G. gilruthi could cause severe pathogenesis by destroying the epithelium of the gastrointestinal tract. The Globidium type of cysts, however, are considered non-pathogenic. In sectioned material and macroscopically, the cysts of G. gilruthi have been seen to have liberated the 'spores'. Evidently, most of them are unable to reach their site of predilection or they fail to establish themselves.

An oocyst or oocyst-like structure in the case of natural infection with G. gilruthi has been reported recently (Tewari & Iyer, 1960). The size of the oocyst is given as 144 x 100 μ and the thick wall of oocyst has been described to be hyaline and eosinophilic. One oocyst, which is unlike any of the known species of Eimeria in goats, was found during the course of present work. It is very difficult to say if the same has any connection with G. gilruthi.

During the course of present work, the Globidial type of cysts were quite common in the abomasum and elsewhere in the gastrointestinal tract. The morphology and histology of these are similar to the classical descriptions of

Flesch (1883), Gilruth (1910), Chatton (1910a), Soliman (1960) and Tewari & Iyer (1960). Whenever these G. gilruthi cysts were found, the tissue phases of Eimeria species, which were present in the intestine, could easily be differentiated, both microscopically and macroscopically.

On the whole the nature of G. gilruthi continues to be doubtful, but at the same time, there is no strong justification for associating it with Eimeria until some more could be known about the nature, biology and life cycle of this parasite in goats.

SUMMARY

SUMMARY

A survey of the species of Eimeria in the goats has been carried out. A total of 243 goats have been examined and eleven species of Eimeria were encountered. Out of these, E. ahsata and E. christenseni are new to the country. The other species are E. arloingi, E. crandallis, E. faurei, E. granulosa, E. hawkensi, E. intricata, E. ninakohlyakimovae, E. pallida and E. parva.

The intensity of infection and the incidence of the different species has been analysed. E. arloingi, E. parva, E. ninakohlyakimovae and E. faurei are the commoner species in the goats. Infection with only one species of Eimeria are not common. The maximum number of species found in a single host is seven. In their seasonal incidence, the parasites are more prevalent during the months of October to December.

The detailed morphology and biometry of all the eleven species of Eimeria has been determined. The sporulation time for all the species under uniform conditions, has been determined. The validity of E. pallida is questionable.

E. faurei and E. ninakohlyakimovae have been successfully transmitted from goats to young lambs. This establishes their lack of host specificity for closely related hosts. E. faurei evidently has two generations of schizogony in self-limited life-cycle. E. ninakohlyakimovae is pathogenic to the young lambs.

The incidence of Globidium gilruthi in the goats has been surveyed. This dubious parasite is quite common in the gastrointestinal tract of the goats and is found in 58.44% of the goats examined. The schizonts and microgametocytes are found all over the small intestine and abomasum. The morphology and histochemistry of the cysts has been described and analysed. The validity of Globidium gilruthi has been discussed. This doubtful parasite has some resemblance with Eimeria species. Certain features of the cysts, like the inclusion of globidial bodies in the "spores", prominent cyst wall and the unknown life-cycle, however, make it difficult to assign this parasite to Eimeria.

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PLATES

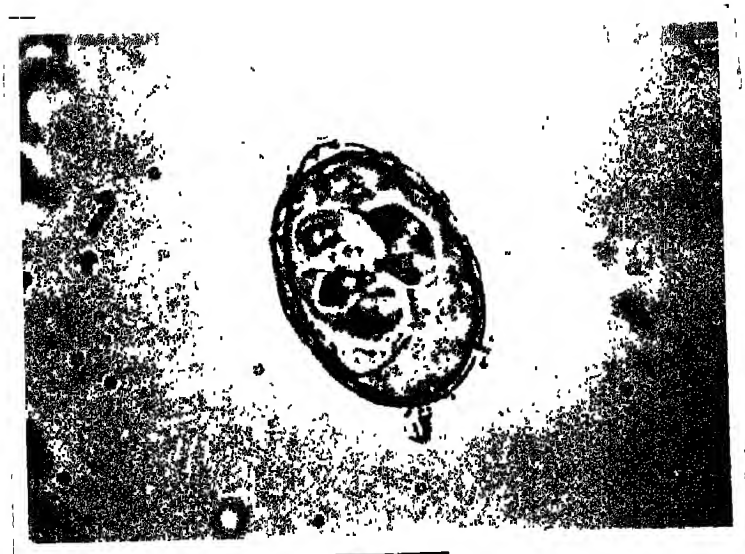

PLATE I

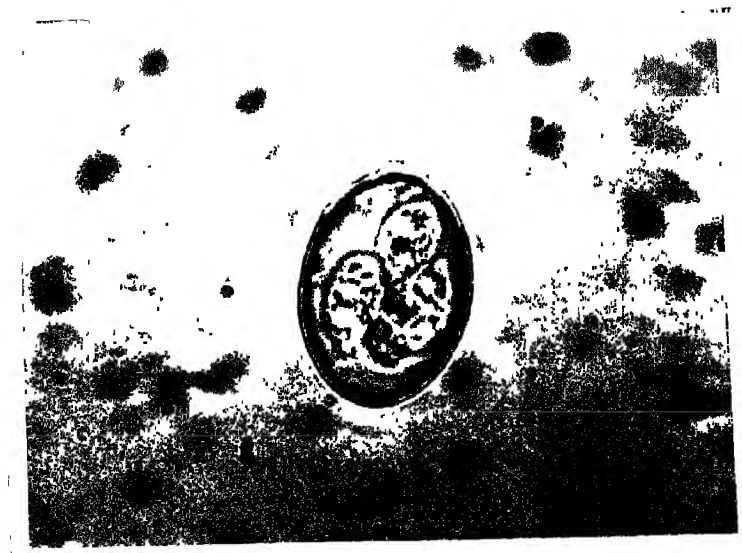

PLATE II

PLATE III

PLATE IV

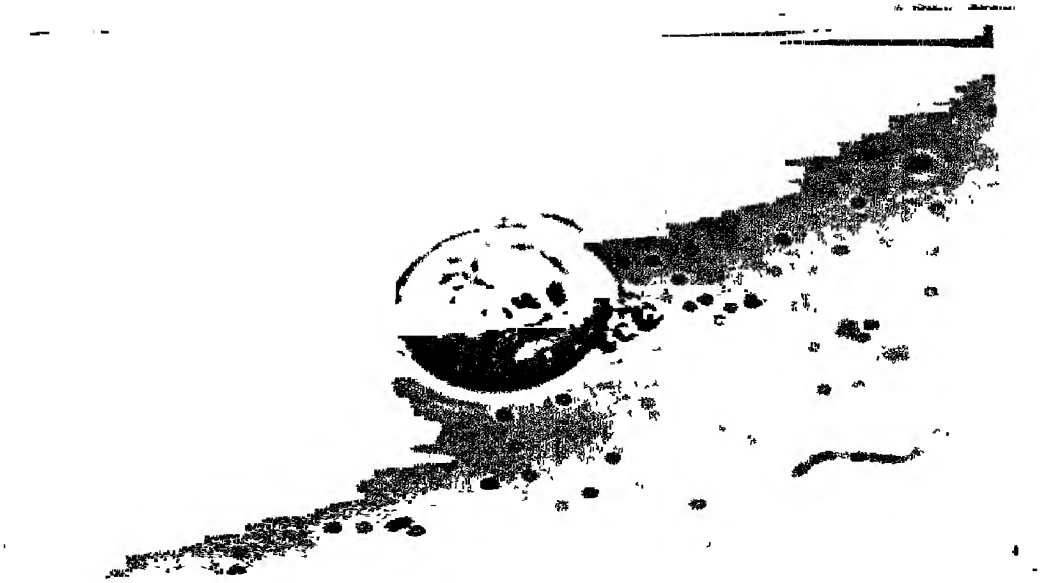

PLATE V

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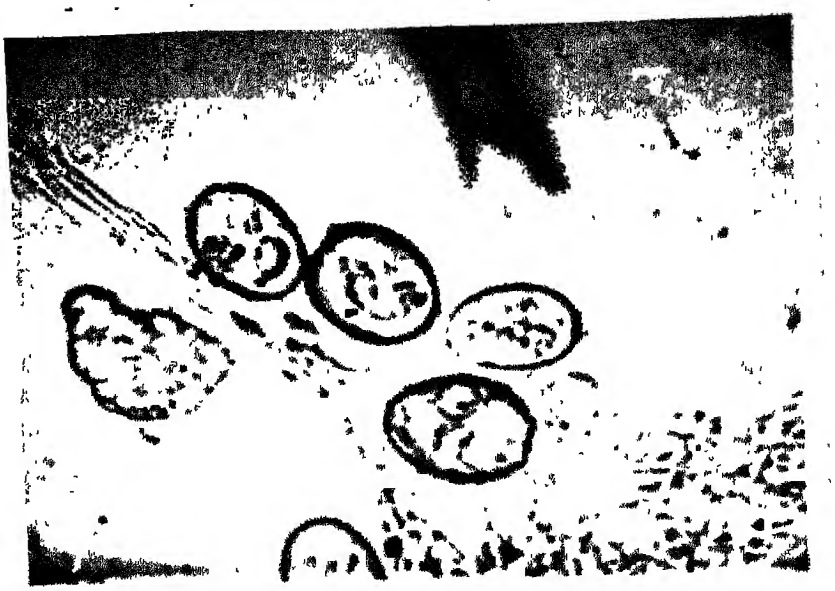

PLATE VII

PLATE VIII

PLATE IX

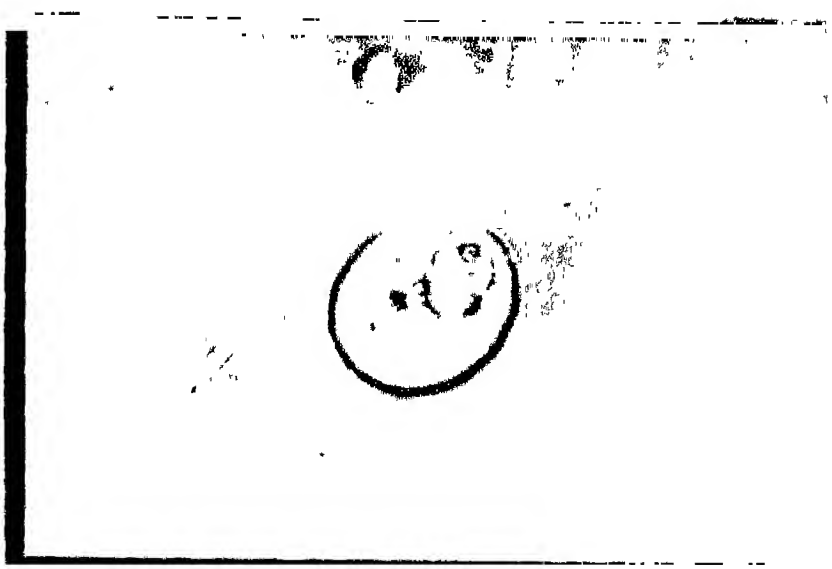

PLATE X

Plate XI Sporulated oocyst of E. parva.
ca. 1100 x

Plate XII T.S. of intestine of lamb experimentally infected with oocysts of E. ninakohlyakimovae. Showing desquamation of the epithelial lining of the villi and the presence of the developmental stages of E. ninakohlyakimovae. Haematoxylin & Eosin.
ca. 110 x

PLATE XI

PLATE XII

Plate XIII Same as Plate XII. gm. 290 x

Plate XIV Same as Plate XII. gm. 480 x

PLATE XIII

PLATE XIV

Plate XV An oocyst suspected to be that of Globidium gilruthi. ga. 660 x

Plate XVI Cross section of Abomasum of goat showing schizont stage of Globidium gilruthi Haematoxylin & Eosin. ga. 480 x

PLATE XV

PLATE XVI

Plate XVII Same as Plate XVI. ca. 1100 x

Plate XVIII Cross section of abomasum of goat showing schizont of Globidium gilruthi in the submucosa. Gomori's trichrom stain. ca. 110 x

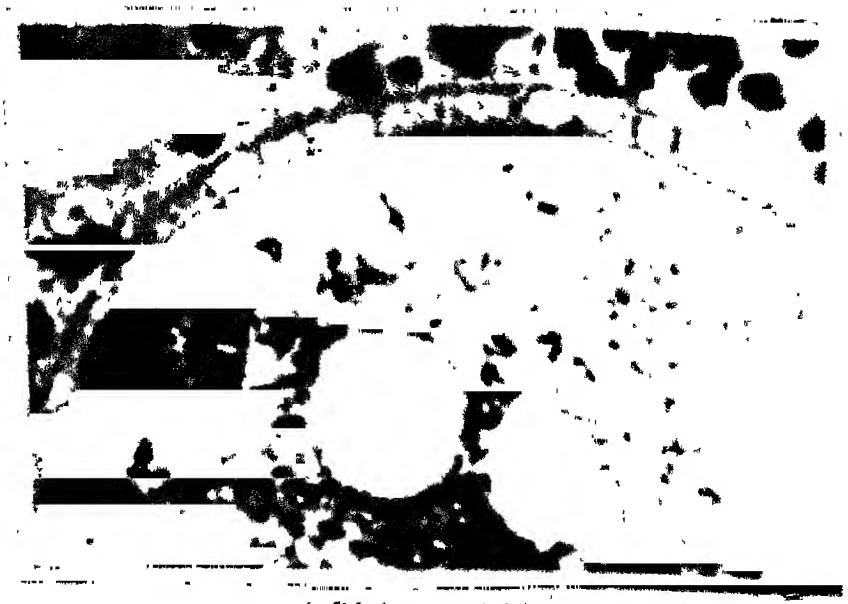

PLATE XVII

PLATE XVIII

Plate XIX Same as Plate XVIII. ca. 480 x

Plate XX Similar to Plate XVIII. Note intensive cellular infiltration. Haematoxylin & Eosin. ca. 110 x

Plate XXI Cross section of abomasum of goat showing a microgametocyte of Globidium gilruthi in the submucosa. Silver impregnation & Van Gieson stain. ca. 1100 x

Plate XXII Similar to plate XXI. Haematoxylin & Eosin. ca. 110 x

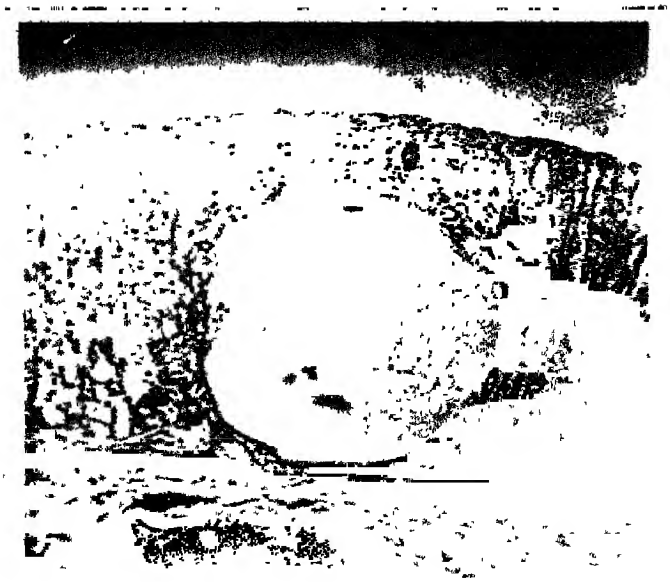

PLATE XXI

PLATE XXII

Plate XXIII Similar to Plate XVIII. Note the discharge of the "spores" from the ruptured cyst. Mallory's phosphotungstic acid haematoxylin. ga. 110

Plate XXIV T.S. of jejunum of goat showing schizont and macrogametocyte of G. gilruthi. Haematoylin & Eosin. ga. 110 x

PLATE [illegible]

PLATE [illegible]

Plate XXV Similar to Plate XVIII. Haematoxylin & Eosin. ca. 480 x

Plate XXVI Similar to Plate XVIII. Haematoxylin & Eosin. ca. 290 x

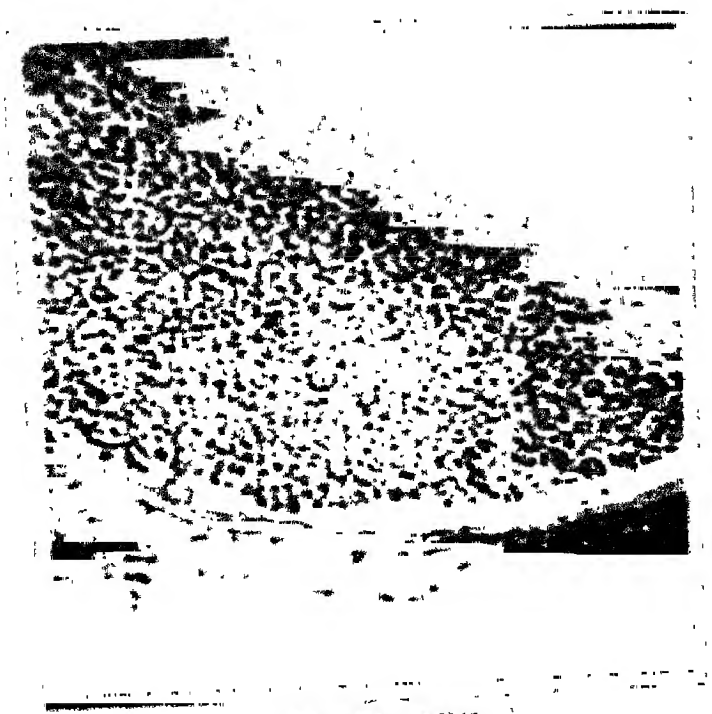

PLATE XXV

Plate XXVII Similar to Plate XVIII. Methyl Green-Pyronin stain. oil. 290 x

Plate XXVIII Same as Plate XXIII. oil. 1100 x

PLATE XXVII

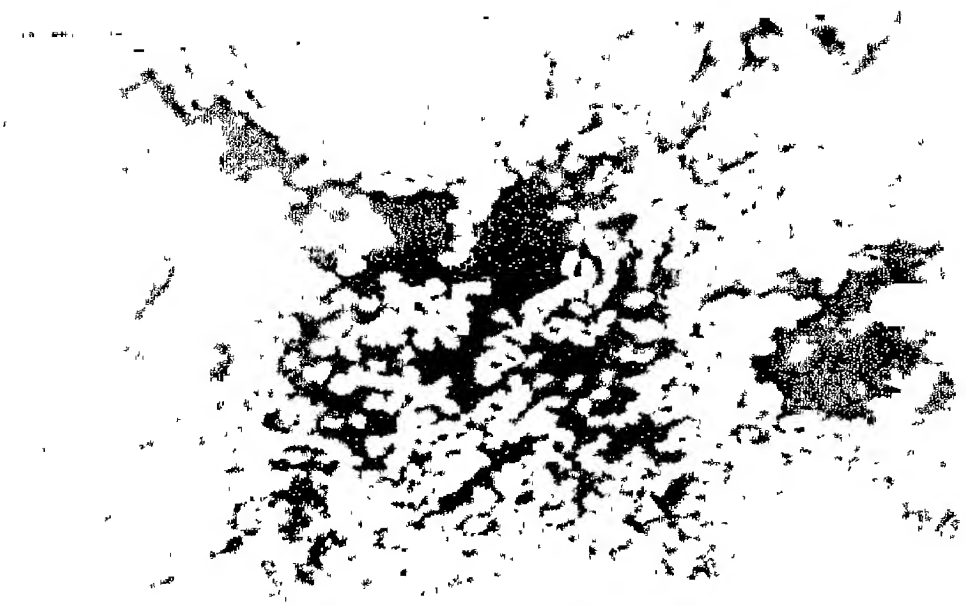

PLATE XXVIII

Plate XXIX Similar to Plate XXI showing leucocytic infiltration. Haematoxylin & Eosin. ga. 1100 x

Plate XXX Similar to Plate XXI showing infiltration with leucocytes and macrophages. Methyl Green-Pyronin. ga. 1100 x

PLATE XXIX

FIGURES

Figures drawn with the aid of Camera Lucida

Figure 1. Sporulated oocyst of *E. christensenl*.

Figure 2. Sporulated oocyst of *E. hawkensi*.

[redacted]